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6077

Sensitization of Rabbits with Neoarsphenamin.

J. W. MU. (Introduced by Louise Pearce.)

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Although it has been demonstrated[1, 2, 3] that guinea pigs can be readily sensitized to neoarsphenamin, Frei[1] reported that he could achieve no hypersensitiveness to this drug in rabbits. In view of the findings of Sulzberger and Mayer[4] that guinea pigs bred in different countries reacted differently to the same brand of neoarsphenamin, experiments have been carried out to determine whether the rabbits bred in Peiping could be sensitized by this compound.

The brand of neoarsphenamin used throughout the work was that prepared by Hoechst Company, Germany. The ampules used for each experiment were from the same batch. 0.15 gm. of the drug was dissolved in 10 cc. of doubly distilled water and the solution diluted to 100 cc. with sterile normal saline. Only freshly prepared solutions were employed.

Observations have been made on 50 native bred adult male rabbits; individual body weights varied from 1350 to 2320 gm. All animals were albinos and the majority were of a delicate build with a slender head and moderately short ears. Thirty rabbits had been in-

[1] Frei, W., *Klin. Wchnschr.*, 1928, **7**, 1026.

[2] Sulzberger, M. B., *Arch. Dermat. and Syph.*, 1929, **20**, 669; 1930, **22**, 839.

[3] Mu, J. W., to be published in *Arch. f. Dermat. u. Syph.*

[4] Sulzberger, M. B., and Mayer, R. L., *Arch. Dermat. and Syph.*, 1931, **24**, 537.

oculated with *Treponema pallidum* from 4.5 to 34 months previously. At the time of the present experiments, only 4 showed active syphilitic lesions, the remainder being in the latent stage of the infection. Twenty-four hours before each experiment, the skin over both flanks was shaved; only ordinary soap was used. In each instance, the right flank was used for the first, and the left for the second injection, both being administered intradermally. The second or "testing" injection was made 4 weeks after the administration of the sensitizing injection. In the case of 8 normal and 12 syphilitic rabbits, 0.1 cc. was used for both the sensitizing and the testing doses. Twelve normal and 18 syphilitic rabbits were sensitized and tested with 0.2 cc. respectively.

Of the 8 normal rabbits sensitized and tested with the smaller amount of neoarsphenamin (0.1 cc.), none had either a flare-up or a hypersensitiveness reaction. Of the 12 syphilitic animals in which the same procedure was used, 1 showed a flare-up reaction and 2 a hypersensitiveness reaction. Of the 12 normal rabbits sensitized and tested with 0.2 cc., only 1 developed a flare-up and hypersensitiveness reaction. But of the 18 syphilitic rabbits sensitized and tested with this dose, 3 revealed a flare-up reaction and 9 a hypersensitiveness reaction. It should be pointed out that these responses were entirely similar in appearance to those previously observed in guinea pigs.[3]

In the last experiment, 5 normal young male albino rabbits were also sensitized and tested with doses of 0.2 cc. respectively. Two showed a flare-up reaction and 3 a hypersensitiveness reaction. These rabbits were from 2 to 2.5 months of age and their weights ranged from 500 to 920 gm.

The results of these preliminary experiments show that rabbits may be sensitized to neoarsphenamin by intradermal injections. It would seem, furthermore, that the incidence of hypersensitiveness is higher in syphilitic than in normal rabbits, but in view of the small number of animals used, this conclusion is only tentative and may be modified by the results of further studies now in progress.

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Regional Variability of Skin Hypersensitiveness to Neoarsphenamin in Guinea Pigs and Rabbits.

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It is well recognized that variations in the intensity and constancy of hypersensitive cutaneous reactions occur in the human subject with respect to different regions of the body. As far as similar variations in animals are concerned, however, few observations appear in the literature. The object of the present study was to ascertain whether different skin areas of guinea pigs and rabbits sensitized to neoarsphenamin would react in a similar or in a different manner to subsequent intradermal injections of this drug.

The experiments included 9 adult male albino rabbits and 12 adult guinea pigs; in the latter animals the skin of the flanks and abdomen was non-pigmented. The neoarsphenamin solution was prepared and the animals were shaved according to the technique described in the preceding report.[1] The rabbits were sensitized and tested for hypersensitiveness with 0.2 cc. and the guinea pigs with 0.1 cc. The injections which were given with a sharp tuberculin needle and a tuberculin syringe calibrated to 0.01 cc., were made as equally deep in the epidermis of the flank and abdomen as possible; the wheals formed were of approximately the same area and depth. The test

TABLE I.

Measurements of Skin Hypersensitiveness Reactions on the Flank and the Abdomen.

No. animal	Guinea Pig Hypersensitiveness Reaction (cm.)		No. animal	Rabbit Hypersensitiveness Reaction (cm.)	
	Flank	Abdomen		Flank	Abdomen
1	1.0 x 1.0		1	1.2 x 1.2	
2	1.0 x 1.0	1.0 x 1.0	2	1.0 x 0.8	
3	1.0 x 0.7		3	1.0 x 1.5	0.5 x 0.5
4	0.8 x 0.8	0.3 x 0.3	4	2.0 x 1.5	1.0 x 1.0
5	1.0 x 1.0		5	1.0 x 1.5	0.5 x 0.5
6	1.0 x 1.0	0.5 x 0.5	6	1.8 x 1.5	0.8 x 0.8
7	1.2 x 1.2		7	1.5 x 1.5	
8	0.8 x 0.8		8	1.0 x 1.5	0.5 x 0.8
9	0.8 x 0.8		9	1.0 x 1.0	
10	1.0 x 1.2				
11	1.0 x 0.7				
12	0.7 x 0.7	0.5 x 0.5			
Average	0.9 x 0.9	0.2 x 0.2		1.3 x 1.3	0.4 x 0.4

[1] Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 781.

for hypersensitiveness was made 5 weeks after the first or sensitizing injection. The reaction was read 24 hours after injection, the width and breadth of the involved skin area being measured.

The results shown in the accompanying table indicate that there was a distinct variation in the reactions on the flank and abdomen in every animal, those on the flank being larger than those on the abdomen. Furthermore, in 8 of the 12 guinea pigs and in 4 of the 9 rabbits, there was practically no reaction in the abdominal area in contrast to the definite flank reaction in each case. Besides the difference in the size of the reaction, most of those on the abdomen were macules, while those on the flank were for the most part papules.

6079

A Case of Hybrid Vigor in the Albino Mus Musculus.

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Two strains of mice, both albino *Mus musculus* and here designated as H T M and E L T M, are known to differ in the number of tail-rings as previously reported (Fortuyn[1]). They also show differences in the number of young per litter which the mother, in the absence of the father, is able to raise to an age of one month. In each strain this has been tested in the offspring of a single pair so as to reduce variability. Table I gives the size of the litters. In 45 first litters of H T M the average number of young which lived

TABLE I.

Mother x father	Litter No.	Total No. of litters	Number of litters, one month old, with the following number of young. 0	1	2	3	4	5	6	7	8	9	10	11
H T M x H T M	1st	45	5	0	4	6	3	9	4	7	6	0	1	
H T M x H T M	2nd	28	2	0	1	1	3	9	2	4	2	2	1	1
E L T M x E L T M	1st	32	18	3	1	4	2	1	2	1				
E L T M x E L T M	2nd	39	19	8	0	2	3	2	3	2				
same mothers H T M x H T M	1st	26	2	0	3	4	1	6	1	4	4	0	1	
same mothers H T M x E L T M	2nd	26	1	0	0	0	1	2	2	7	9	3	1	
same mothers H T M x E L T M	1st	26	1	0	2	2	4	3	4	4	3	3		
same mothers H T M x H T M	2nd	26	2	0	1	1	3	8	2	4	2	1	1	1
same mothers E L T M x H T M	1st	15	2	2	2	1	0	2	3	1	1	0	0	1
same mothers E L T M x E L T M	2nd	15	6	4	0	1	1	1	0	2				

[1] Fortuyn, A. B. D., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 543; *Genetics*, 1931, **16**, 160.

with the mother to an age of one month is 4.75 ± 0.48 (mean error). For 28 second litters this figure is 5.60 ± 0.48. In 32 first litters of E L T M the average number of young, one month after birth, is only 1.53 ± 0.37, in 39 second litters it is 1.74 ± 0.37. The differences with their mean errors amount to 3.22 ± 0.53 for the first litters and 3.86 ± 0.60 for the second, and are therefore statistically significant in both cases. This difference in fecundity is explained by the fact that in H T M only 5 first litters and 2 second ones are completely destroyed by the mother, whereas in E L T M these figures are 18 and 19. Moreover, in those cases where the litter is not completely destroyed, more young survive in H T M, which may have litters of 10 and 11 young than in E L T M which never had litters of more than 7 young. No observations have been made on the question of whether death occurs shortly after birth through the inability of the young to suck, or because the mother has no milk, or kills the young independently from either condition.

These 2 strains were crossed. In the first instance 26 H T M females were given H T M males as fathers of their first litters and E L T M males as fathers of their second litters. Another group of 26 H T M females were crossed with E L T M for the first litters but mated with H T M for their second litters. All mothers were of approximately the same age. All the resulting litters, therefore, are as strictly comparable as possible. The average number of surviving offspring at the age of one month for the pure H T M first litters is 4.92 ± 0.50 and for the second litters 5.50 ± 0.50. For the hybrid litters the average number is 5.53 ± 0.45 for the first and 7.07 ± 0.38 for the second litters. For the first group of mothers with pure first litters and hybrid second ones, the difference in the number of surviving young is 2.15 ± 0.62 and therefore significant. In the second group the advantage which the second litter usually has over the first one is completely balanced by the fact that the first litter is hybrid and the second one pure.

For E L T M mothers less complete figures are available. 15 E L T M mothers raised on the average 4.20 ± 0.80 young per litter to the age of one month in their first litters when H T M mice are the fathers, but these same mothers raised only 2.00 ± 0.63 young in their second litters when E L T M mice were the fathers. If the hybrid first litters are compared with the pure first litters of the strain in general again a significant difference is found (2.67 ± 0.88). Another remarkable fact is that, whereas the largest observed litters of E L T M were of 7 young, among the 15 hybrid litters one contained 8 and one 11 young.

So-called hybrid vigor, or heterosis, usually finds its expression in physical strength, in fertility, or in longevity, in which characters the hybrids surpass both parent strains. In the case of the mice described above no difference in size has been noticed and about the duration of life nothing is known, but there is no doubt that a cross between the 2 strains gives more viable young than either one of the pure strains. The birthrate in this case has not been studied, because, especially in the case of E L T M where the mothers destroy so many young, it would require the closest observation to state accurately how many young are born. On the other hand the observation as to how many survive with the mother one month after birth is easily made. Hybrid vigor certainly does not occur in all crosses between mice and the term is used for a variety of phenomena. Most often the explanation is lacking but sometimes hybrid vigor is explained by the fact that genetical factors which promote each other are combined in the hybrid. A more complete analysis will be necessary to understand the case reported here.

6080

A Simplified Technique for the Study of Pneumococcus Growth Inhibition.

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Various techniques have been employed to determine the pneumococcidal power of serum, plasma or whole blood of naturally or artificially immune animals. Robertson and Sia[1] used serum-leucocyte-cocci mixture placed in small paraffin corked tubes and agitated during incubation in a manner simulating the circulating blood. The procedure is complex and time-consuming. Bull and Tao[2] claimed that whole blood prevented from coagulation by sodium citrate served just as well as the serum-leucocyte mixture in demonstrating the pneumococcidal property of the sera of chicken and immune rabbits and that agitation was not necessary as shown to be important by the former workers. The present report deals with the results obtained while looking for a simplified technique of the original method.

[1] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **40**, 467.

[2] Bull, C. G., and Tao, S. M., *Am. J. Hyg.*, 1927, **7**, 648.

Since the citrated whole blood is so easy to obtain and has been claimed to give satisfactory results we repeated the experiments according to the details set forth by Bull and Tao. 1% by volume of saturated sodium citrate (Merck) solution (determined to be nearly 100%) was used. A virulent Type I pneumococcus was grown in broth for 16 hours. At the same time the original method of Robertson and Sia was carried out as control. Many experiments were done at different times and the results are summarized in Table I. It will be noticed from columns A and C that while the

TABLE I.

Comparison of pneumococcus growth inhibition tests by the method of Bull and Tao with that of Robertson and Sia.

Dilution of Pneumococci	Citrated Whole Blood						Serum-leucocyte Mixture	
	A				B		C	
	Rabbit	Cat	Dog	Chicken	Dog	Chicken	Cat	Dog
Undiluted			+	+				+
1:10			+	+		+		+
1:100		+	+	+	+	0	+	+
1:1,000		+	+	+	+	0	+	+
1:10,000		+	+	+	+	0	0	0
1:100,000		+	+	+	0	0	0	0
1:1,000,000	+	+	+	+	0	0	0	0
1:10,000,000	+	+	+	+	0	0	0	0
			Plasma Control				Serum Control	
1:1,000,000		+	0	+	0	0	+	+
1:10,000,000		+	+	+	0	0	+	+

+ = Growth in 48 hours by smears. 0 = No growth.

original method clearly demonstrates the pneumococcidal property of the blood of the resistant animals, this property failed to appear in the citrated blood of the same animals. On the other hand, as shown in column B, it was found in a few instances that there was no growth in the control tubes with the citrated plasma of the naturally resistant animals as well as the citrated whole blood series in the tubes seeded with the higher dilutions of the culture. This result might be due to pneumococcidal power of the blood, but the absence of growth in the plasma led us to suspect the inhibitory effect of sodium citrate upon the growth of pneumococci. Experiments were then done to determine this effect of the anti-coagulant, and the results are shown in Table II. Similar experiments with blood and plasma of cat and chicken gave identical results with that of the dog. Three facts present themselves. First, sodium citrate in the strengths tested had a definite detrimental effect upon the organisms. The margin of safety is so very small that a difference of even 0.1% brought out a marked change in the reaction of dog's blood. Second, with equal strength of sodium citrate, pneumococcus

TABLE II.

Effect of varying amounts of sodium citrate upon the growth of pneumococci in whole blood and plasma of rabbit and dog.

	Sodium Citrate %	1.0	1.0	1.1	1.2	1.2	1.3	1.4	1.4	1.5
	Dilution of pneumococci	Blood	Plasma	Blood	Blood	Plasma	Blood	Blood	Plasma	Plasma
Rabbit	1:100				+	+		+	+	0
	1:1,000				+	+		+	+	0
	1:10,000				+	+		+	+	0
	1:100,000	+	+	+	+	+		+	0	0
	1:1,000,000	+	+	+	+	+		+	0	0
	1:10,000,000	+	0	+	+	0		0	0	0
Dog	1:10	+	+	+	+		+	Few		+
	1:100	+	+	+	0		0	0		0
	1:1,000	+	+	0	0		0	0		0
	1:10,000	+	0	0	0		0	0		0
	1:100,000	+	0	0	0		0	0		0
	1:1,000,000	+	0	0	0		0	0		0
	1:10,000,000	+	0	0	0		0	0		0

has a better chance of growth in the whole blood than in the plasma of the same animal. Third, sodium citrate in the blood or plasma of resistant animals exerts a greater injurious effect upon pneumococci than in blood or plasma of a susceptible animal. Cheer[3] demonstrated that sodium citrate in strengths of 0.8 and 1.0% exerted a growth inhibitory action upon pneumococci. Therefore, it seemed to us that the pneumococcidal power of the citrated blood as reported by Bull and Tao could be explained by the inhibitory effect of sodium citrate upon the growth of organisms themselves rather than by the interaction of serum and leucocytes. And similarly, it would also explain the fact that agitation during incubation was claimed to be not necessary. If they had controlled their experiments with citrated plasma as we did in ours, they might have arrived at a similar interpretation of their results. We also believe that sodium citrate in a strength too weak to affect the organisms would injure the leucocytes when they come into contact with each other for 24-48 hours as this test requires. This may explain our results shown in column A of Table I where there was growth in every tube of the citrated blood of resistant animals as well as that of susceptible animal.

The modification of the original method consists of either of the following:

A. "Reconstituted blood": A desired amount of blood is sterilly withdrawn and divided into 2 portions. One portion is allowed to

[3] Cheer, S. N., *J. Imm.*, 1930, **18**, 187.

coagulate to give serum. The other portion is well mixed with 1-2 parts of 1% sodium citrate in normal saline, centrifuged and the supernatant fluid discarded. The cells are then washed once in gelatin salt solution and the washings discarded. Enough homologous serum is finally added to make up to the original volume of the blood. This non-coagulable reconstituted blood is deprived of its fibrin and spared of its normal active leucocytes since they are in contact with citrate for only a very short time. B. "Defibrinated blood": This is accomplished by shaking a sample of blood thoroughly in a flask with glass beads. The results with the defibrinated blood have been very satisfactory and in a way confirm the contention of Todd[4] and Ward[5] that complete defibrination does not

TABLE III.
Growth inhibition test by "Reconstituted" and Defibrinated Blood.

	Dilution of pneumococci	Reconstituted blood	Serum control	Defibrinated blood	Plasma control
Rabbit	1:100,000	+		+	
	1:1,000,000	+		+	+
	1:10,000,000	+		+	+
Dog	1:10	+		+	
	1:100	+		+	
	1:1,000	+		+	
	1:10,000	0		0	
	1:100,000	0		0	+
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	
Cat	1:10	+		+	
	1:100	+		+	
	1:1,000	+		+	
	1:10,000	0		0	
	1:100,000	0		0	+
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	
Chicken	1:10	+		+	
	1:100	+		+	
	1:1,000	0		+	
	1:10,000	0		0	
	1:100,000	0		0	+
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	
Human*	Undiluted	+		+	
	1:10	+		+	
	1:100	+		0	
	1:1,000	0		0	
	1:10,000	0		0	
	1:100,000	0		0	
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	

*Type I pneumonia convalescent. Blood taken 9th day after crisis.

[4] Todd, E. W., *Brit. J. Exp. Path.*, 1926, **7**, 368; 1927, **8**, 1.

[5] Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675.

alter the phagocytic activity of the leucocytes. The number of leucocytes present in the defibrinated blood is apparently not markedly reduced. In one or two instances actual counts on the different lots of the blood were made. In that of dogs the count varied from 11,800 to 13,600 per c.mm.

In actual tests, 0.5 cc. of blood (0.4 or 0.3 cc. have been found to be quite satisfactory) prepared according to either A or B is placed in each of a series of small tubes, and 0.1 cc. of the various dilutions (in broth) of pneumococcus is added. The tubes are corked and agitated according to the method of Robertson and Sia. Rotation was found essential. The results obtained by these 2 modifications (Table III) were so consistently good and they compared so favorably with those of the original method that we consider it worthwhile to propose their use as a simplified technique. The method has been used to measure the potency of an immune serum and to detect the pneumococcidal power of human convalescent blood. It was found applicable. While the "reconstituted blood" offers one the means of studying serum and leucocytes from different individuals the defibrinated blood has the distinct advantage of being the simpler. However, in view of some inhibitory effect of dog plasma upon the growth of pneumococci* the latter modification is to be preferred only in selected instances.

Summary. Citrated whole blood has been shown to be inadequate for the study of pneumococcus growth inhibition on account of the detrimental effect of sodium citrate upon the organisms on the one hand, and upon the leucocytes on the other. A modification of the original method of Robertson and Sia is proposed. Because of the simplicity and the satisfactory results it gives, this technique may find a wider application for similar studies with other organisms.

* The inhibitory effect of dog plasma upon the growth of pneumococci will be taken up in detail in a further communication.

6081

Effect of Type II Pneumococcus Soluble Specific Substance on Infectivity of Pneumococcus Type II.

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One of us previously showed that the pneumococcus soluble specific substance exerted a specific antiphagocytic action on the blood *in vitro*.[1] This finding has since then been confirmed by others.[2, 3, 4] *In vivo* attempts, on the other hand, have not so far given similar striking results. By injecting Type II soluble substance together with an attenuated Type II pneumococcus in mice, Felton and Bailey[5] found that it exhibited an antagonistic effect on the defense of the animals or an actual increase in the virulence of the organism from 10-100 folds. We wish to record the results of a study along similar lines but with more striking effects.

The soluble specific substance employed in this work was prepared from a Type II pneumococcus according to the method of Heidelberger and Avery.[6] The chief organism used was a strain of a typical Type II pneumococcus, highly virulent for white mice, but relatively avirulent for rabbits.

Preliminary experiments with a modified method[7] for demonstrating growth-inhibitory and pneumococcidal action of the blood before and after the intravenous injection of Type II pneumococcus soluble substance, showed that while rabbit blood normally was capable of destroying large numbers of Type II pneumococci, blood taken after the injection of the substance had entirely lost this property.

The effect of the intravenous injection of the soluble substance on the course of pneumococcus bacteremia in rabbits was next studied. It was found that when a sublethal dose of the Type II pneumococcus was injected intravenously into normal rabbits, the

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1 Sia, R. H. P., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 262; *J. Exp. Med.*, 1926, **43**, 633.

2 Wadsworth, A. B., and Sickles, G. M., *J. Immunol.*, 1927, **14**, 321.

3 Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675.

4 Alston, J. M., Galbraith, G. R., and Stewart, D., *J. Path. and Bact.*, 1930, **33**, 845.

5 Felton, L. D., and Bailey, G. H., *J. Inf. Dis.*, 1926, **38**, 131.

6 Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1923, **40**, 301.

7 Wu, C. J., and Sia, R. H. P., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 786.

organisms quickly disappeared from the blood stream; whereas in rabbits receiving soluble substance and similar amounts of pneumococcus culture, an increase in the bacteremia ensued and frequently terminated in the death of the animals. In the light of Tillett's[8] work, the course of bacteremia in these 2 series of animals may be interpreted to mean that the pneumococcus soluble specific substance causes an increased virulence of the organism. On the other hand, the injection of a culture of a "Rough" variant derived from Pneumococcus Type II after the animals had received similar amounts of the soluble substance failed to produce similar effects.

Experiments with the injection of Type II pneumococcus culture broth instead of the purified soluble substance gave practically identical results as the latter.

Further study on the effect of the injection of Type II pneumococcus soluble substance on the mortality rate of the rabbits which were subsequently infected with the homologous organism, suggested an increase in the susceptibility of the animals or an increased virulence of the organism by 10,000-100,000 times. Similar experiments in which the infecting organism was substituted by a "Rough" variant of a Type II pneumococcus again failed to produce such action. None of these latter animals even seemed to have suffered in any way by such injections.

6082

Use of Antipneumococcus-Serum-Agar for the Identification of Pneumococcal Types.*

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It is generally recognized that Type III pneumococci grow on blood agar plates in large mucoid transparent colonies. This peculiar growth characteristic of the organism has frequently rendered its isolation from plates quite simple. The identification of the other types of pneumococci by the appearance of their colonies, on the other hand, is beset with great difficulties, if not an impossibility. Such identification usually requires agglutination tests carried out with cultures obtained from the individual colonies.

[8] Tillett, W. S., *J. Exp. Med.*, 1927, **45**, 1093.

* We are indebted to Dr. A. B. Wadsworth for the antipneumococcus horse serum employed in this investigation.

In a further study on the *in vitro* transformation of pneumococcal types, where at times the presence or absence of 2 different types of pneumococci, *e. g.*, Type I and Type II, is to be determined, numerous colonies would have to be picked and typed with known immune sera. It was felt, therefore, that a simpler method of identification would save considerable amount of time and material, and render such a study easier technically.

Virulent pneumococci elaborate, during their growth, a soluble specific substance which gives specific precipitation with homologous immune serum. Therefore, it was thought reasonable that if type specific antipneumococcus serum be added to the agar medium, any homologous soluble specific substance elaborated around each colony of pneumococcus might give these colonies a different appearance.

Plates were accordingly made with plain beef infusion agar pH 7.8, to which 1% dextrose and varying amounts of Type I antipneumococcus serum were added, and a culture of Type I pneu-

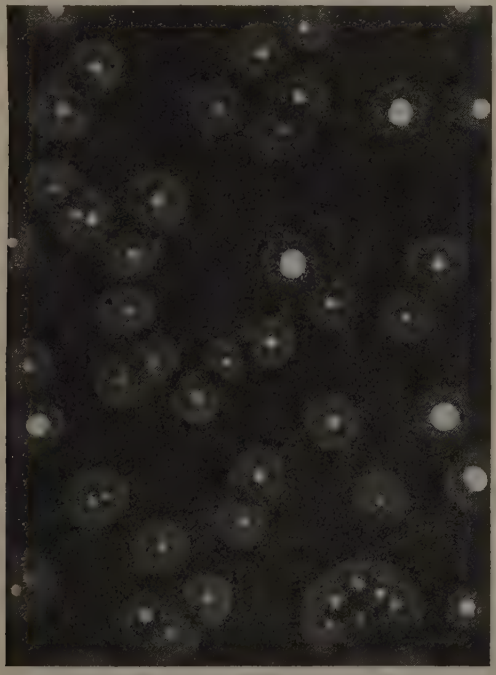

FIG. 1.

Type I pneumococcus colonies grown in Type I antipneumococcus-serum-agar. Showing annular opacities surrounding each colony. × 3.

mococcus was used to streak the plates. However, the results were entirely disappointing. The colonies on all the plates revealed no distinguishing features differentiating them from those grown on plain agar plates.

Poured plates were next employed, and this procedure gave the desired results. With Type I, II or III antipneumococcus serum added to the agar, the homologous organism grown in the depths of the medium after incubation showed annular opacities with a very definite outer margin, surrounding each colony. This was best seen by viewing the plate with illumination from the side on a dark background, and was usually large enough so that it could be seen with the naked eye (see Fig. 1). Colonies that had grown at the bottom or at the surface of the agar tended to spread out, forming large opaque colonies which may be mistaken for the annular opacity just described; therefore, due precaution should be taken when examining these plates. The formation of the annular opacity around the colonies has been found to be strictly type specific.

The amount of antipneumococcus serum to be employed is of great importance. The higher the precipitin titer of an immune serum, the smaller the amount of serum is to be added to the agar. Results similar to the so-called inhibition zone[1] have been observed, in which smaller amounts of immune serum yielded good results while larger amounts negative or poor results. With the diagnostic antipneumococcus sera obtained through the courtesy of Dr. A. B. Wadsworth of the New York State Department of Health, the optimum amounts in the medium have been found to be as follows: For Type I, 3-4%; Type II. 5-6%; and Type III, 1-2%. It is recommended, however, that the optimum amount for each lot of immune serum be tested prior to the use of the immune serum-agar medium.

The length of incubation of the inoculated plates is also of importance. Twenty-hour incubation has been found to be highly satisfactory. Shorter periods of incubation show the annular opacity poorly, while with longer incubation, these annular opacities became larger and finally coalesced, thereby giving rise to a homogeneous clouding of the agar, and defeated the purpose of the medium.

The method now adopted is as follows: 0.3 cc. antipneumococcus serum Type I (or the corresponding optimum amount of immune serum of the other types) is placed in a Petri dish (100 x 10 mm.). 0.5 cc. 20% dextrose solution is also placed in the same Petri dish

[1] Morgan, H. J., *J. Immunol.*, 1923, **8**, 449.

but not allowed to be mixed with the immune serum. A small loopful of a 1:100 dilution of an 18-hour pneumococcus culture is then inoculated into the plate by washing it in the dextrose solution. 10 cc. molten beef infusion agar pH 7.8 cooled to 40-42°C. is then poured and the contents of the plate mixed thoroughly. The plate is then incubated for 20 hours at body temperature, and then examined. Colonies surrounded by annular opacity as described above indicate the same type of pneumococcus as that of the immune serum used.

Working with such a method, mixed cultures of Type I and Type II pneumococci have been rapidly identified and again grown in pure cultures. These cultures have been found to retain their original characteristics.

Summary. A medium consisting of 1% dextrose beef infusion agar pH 7.8 and type specific antipneumococcus serum is presented. Pneumococci of the homologous type when grown in the depths of the immune serum-agar plate develop well defined annular opacity surrounding each colony. The development of the annular opacities has been found to be strictly type specific.

6083

Action of Ephedrine on Peripheral Blood Vessels as Observed in "Transparent Chamber Preparations".*

HSIANG-CHUAN HOU.

From the Department of Pharmacology, Peiping Union Medical College.

Six rabbits with transparent chambers introduced into the ears according to the Sandison[1] technique were studied. The blood vessels studied included both preformed, and newly formed ones of from 10 days to 4 months old. The reaction to ephedrine of these various types of vessels was essentially the same.

Ephedrine hydrochloride in 0.1% to 1.0% solutions in 0.9% saline was introduced by intravenous injection into the marginal vein of the unobserved ear or the saphenous vein of the leg and continuous observation with the microscope under low power was made during the first half hour after the injection.

* This study was started at the Marine Biological Laboratory at Woods Hole, Mass., and a preliminary note was communicated to Dr. K. K. Chen[2] in 1928, to whom the writer is indebted for one of the samples of ephedrine hydrochloride used.

[1] Sandison, J. C., *Am. J. Anat.*, 1928, **41**, 447.

Ephedrine in doses from 0.1 to 0.8 mg. (0.1% solution) injected into the blood stream did not produce a change in the vessels of the ear chamber. A slight narrowing of the central artery and a slowing of the circulation in the vascular bed was observed when the amount injected was 1 mg. (1% solution). The flow in a few capillaries became reversed, while in a few others it was accelerated, although the calibre of these showed no changes. The effect took place in a half to one minute after the injection and disappeared in about 5 minutes.

With a dose of 2 mg. (1% solution) the constriction of the central artery was more marked. The wave of constriction started from the central portion and travelled toward the periphery. This appears to differ from the effect exerted by epinephrin, which usually produced a wave starting from the opposite direction, namely from the periphery to the center. The arterioles, venules and capillaries again showed no changes in the size of the lumen. A slowing or reversal of the flow in a number of these minute vessels was apparently secondary to the changes in the arteries.

Following the primary constriction of the central artery there was a series of relaxation periods alternating with constriction periods. These effects lasted about one-half hour. At first the relaxation phase was very short but gradually it was prolonged until the normal condition was reestablished, when there was observed 2 to 3 short constrictions in the central artery in the course of an hour.

With larger doses of ephedrine, 4 to 5 mg. (1% solution), the effect was more intense. The lumen of the central artery became obliterated immediately after the injection and this constriction persisted for 3 to 4 minutes. The subsequent relaxation and constriction continued in rapid succession for an hour before a normal circulation was resumed. A number of the smaller arteries also showed rings of constriction at points of bifurcation during the first 20 minutes.

No active constriction or dilatation was noted in the arterioles, capillaries, or venules. This fact again differs from that observed with epinephrin which in moderate doses produced a marked constriction of the arterioles.

These findings confirm those described by Chen[2] for the circulation of the frog's tongue, web, mesentery and kidney in showing that ephedrine does not directly affect the extremely peripheral circulation.

[2] Chen, K. K., and Schmidt, C. F., *Medicine*, 1930, **9**, 1.

Missouri Section.

St. Louis University School of Medicine,
Wednesday, March 9, 1932.

6084

Are Seasonal Variations of Thyroid Gland Dependent Upon Corresponding Variations in Anterior Pituitary?*

LOUIS T. BYARS, HILDA FRIEDMAN, WALTER J. SIEBERT AND LEO LOEB.

From the Department of Pathology, Washington University School of Medicine, St. Louis.

Seidell and Fenger[1] observed very striking seasonal fluctuations in the amount of iodine in the thyroid glands of hogs, sheep and beef, more being present and the gland being smaller during the summer than during the winter season. Fenger[2] confirmed these results. Martin[3] working with sheep glands arrived at similar conclusions. Loeb[4] in studying the compensatory hypertrophy of the thyroid gland of the guinea pig noted that this hypertrophy was considerably less in the summer than in the winter months.

We may then assume that during the colder periods of the year the thyroid gland is much more active, perhaps owing to the fact that more demands are made on this organ than during the warmer season. As a result of the lessened need for thyroid hormone during the summer months, iodine, instead of being used in the metabolism of the gland is stored up in the acini.

It has now been established that the functional state of the thyroid gland is to a marked extent influenced by a hormone of the anterior pituitary. Following previous observations of Allen,[5] Smith,[6] and

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

1 Seidell, A., and Fenger, F., *J. Biol. Chem.*, 1912, **13**, 517.

2 Fenger, F., *Endocrin.*, 1918, **2**, 98.

3 Martin, N. H., *Pharm. J.*, 1912, **89**, 144.

4 Loeb, Leo, *J. Med. Res.*, 1920, **41**, No. 4.

5 Allen, B. M., *Anat. Rec.*, 1917, **11**, 486.

6 Smith, P. E., *Anat. Rec.*, 1917, **11**, 57.

Uhlenhuth and Schwartzbach[7] in amphibian larvae, Loeb and Bassett[8] observed that in mammals (guinea pigs) injections of extracts of the anterior pituitary cause a marked hypertrophy and hyperplasia of the acinus cells of the thyroid, a liquefaction and absorption of colloid, indications of an increased hormone action (loss of weight), therefore, in general, changes corresponding to those observed in Graves disease. To these changes corresponded a marked rise in basal metabolism under the influence of anterior pituitary extracts. (Siebert and Smith.[9])

Under these conditions it was conceivable that the seasonal variations in the thyroid gland were due to corresponding variations in the activity of the anterior pituitary. We wished, therefore, to determine whether such seasonal changes could be established in the activity of this gland.

For this purpose we collected separately anterior pituitary glands of cattle during the winter months (1930-31) and during June, July, August and September (1931). We obtained thus 5 different samples of anterior pituitary powder which were kept in the refrigerator and the strength of which was compared by us at the same time by preparing and injecting extracts into young female guinea pigs in the manner previously described by Loeb and Bassett.[10]

Intraperitoneal injections of 0.5 cc., 1 cc., and 2 cc., of the winter, and of the June, July, August and September extracts were given respectively to 2 groups; one being examined after 4 injections and the other group after 6 injections given on successive days. In addition, doses of 0.3 cc. were given for 4 days. The size of the thyroids thus obtained was then compared and this was followed by a microscopic examination of changes in size and shape of the acini; the amount and consistency of the colloid present in the acini, the height and size of the acinar epithelium, number of mitoses and the presence of phagocytes in the remnants of the colloid. In all cases, typical effects previously described were noted in these thyroid glands and no significant differences were found between the effectiveness of the various extracts.

We then compared the effects of the winter, June and August material on the basal metabolism of guinea pigs in the manner described by Siebert and Smith.[9] All these extracts were found ac-

[7] Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, **5**, 1.

[8] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

[9] Siebert, W. J., and Smith, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 622.

[10] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

tive; the August material happened to be the most active one, while the winter and June material were about of equal strength.

Conclusions. We may conclude that seasonal variations corresponding to those which have been observed in the mammalian thyroid gland could not be established in the case of the anterior pituitary of cattle. While these investigations do not absolutely exclude the possibility that cyclic changes of a more subtle kind may occur in this organ, they render them at least very improbable. It is, therefore, probable that the seasonal variations in the thyroid gland are not determined by primary changes of an annual cyclic character in the anterior pituitary, but that they are due to climatic differences during the seasons of the year, and that the effects of these differences are transmitted to the thyroid gland either directly from the periphery or indirectly through the mediation of other organs. The seasonal changes in the thyroid gland apparently correspond to differences in the need of thyroid hormone at different times of the year. Owing to a diminished need of thyroid hormone on the part of the organism during the summer months, after extirpation of the greater part of the thyroid the remaining part of the gland responds less actively with compensatory hypertrophy during this time of the year.

6085

Simple Method for Obtaining Antisera in a Dry State.

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Several reports deal with the preparation of dry antisera. Evaporation *in vacuo* and precipitation in the cold with alcohol or acetone are the methods most used. According to reports dealing with the latter method, the low temperature has been assumed to be the factor preventing denaturation and loss of antibody activity. We have found that the concentration of the organic precipitant is an equally important factor. There exists a critical concentration of the organic solvents methyl, ethyl and propyl alcohol and acetone, in the range of 60% to 75% concentration at which denaturation of serum proteins is maximal. As the concentration is increased from about 75% the degree of denaturation is decreased until at final concentrations of 90% or above serum proteins can be precipitated

by these organic solvents at room temperatures without loss in solubility or antibody activity.

Applying this phenomenon the following method has been used to prepare dry immune sera. To 10 or more (but not less) volumes of acetone add slowly with shaking one volume of the serum. Collect the precipitate on a filter, wash once with acetone followed by 3 washings with anhydrous ether, the precipitated mass being stirred with a wooden spatula after each ether addition. Approximately 5 volumes of ether to each original volume of serum is required for each washing. The final white mass is spread out on the filter paper and placed in the 37°C. incubator for about one hour. The resulting dry mass is readily pulverized with the wooden spatula to an extremely light, white, fluffy powder. This powder is slowly (due to slow wetting) though completely soluble in distilled water or physiological sodium chloride solution. There has been detected no loss in agglutinating activity, hemolytic activity or antitoxin content.

Absolute ethyl alcohol may be substituted for acetone in the above procedure. Ninety-five percent alcohol may also be used but 19 volumes of alcohol to one of serum are then necessary so the final alcohol concentration does not fall below 90%. It is essential to use anhydrous ether. Extraction with U.S.P. or anesthesia ether yields a final product that is granular and slightly brown in color. Even this product, however, is completely soluble.

The final product appears to be dissolved a little more quickly if 95% or absolute alcohol is substituted for the acetone used for washing in the method given above. The order in such a case is acetone precipitation, alcohol extraction, followed by 3 ether extractions.

The above methods of precipitation and washing have been found to be satisfactory at all temperatures below 35°C. Following precipitation the serum proteins must not be left in contact with the organic solvent longer than about 4 hours if the final product is to be completely soluble.

6086

Occurrence of Hemophilus Influenzae in Throats of Polar Eskimos.*

J. RALPH WELLS AND EVELYN DIXON. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology, Washington University Medical School, St. Louis.

In the fall of 1930, we began a study of the origin of immunity to diphtheria among the Central and Polar Eskimos and of the nature of their throat flora. The materials were collected from 115 Eskimos by Dr. Peter Heinbecker during the late summer of 1930 and in a report soon to be published[1] we have stated that no hemophilic organisms were found. At the beginning of the current year we continued this study with a new supply of approximately twice the number of throat cultures and with additional safeguards against the dying off of these more delicate organisms, in case they should be present in the original materials.

From this latter group of throat cultures, 18 strains of Gram negative hemophilic bacilli possessing the accepted characteristics of *Hemophilus influenzae* were isolated, and this preliminary report is made to supplement and correct our statement already referred to, while a more detailed and complete description of these strains will appear later in the *Journal of Infectious Diseases*.

All these strains refused to grow on plain agar and only occasionally produced scanty growth on infusion agar to which filtered raw tomato juice had been added. On the contrary, all strains grew well on fresh blood agar and still better on this medium after the addition of tomato juice. The "satellite" phenomenon was very marked with many of the cultures, especially around colonies of hemolytic staphylococci, thereby strengthening the evidence that they required both a vitamine-like factor and hemoglobin for growth. Several strains were hemolytic and all strains tested showed nitrate reduction, indol production, ability to ferment dextrose, and bile solubility.

Of 5 strains saved for a more complete study, 4 were hemolytic, capsulated, and agglutinable by type "A" serum of Pittman.[2] The other strain was non-hemolytic, non-capsulated, and non-agglutinable at 37° in this serum, thereby corresponding to the "R" strains of the latter investigator.

* This work supported in part by the Science Research Fund of Washington University.

[1] *J. Infect. Dis.*, 1932, **50**, 281.

[2] Pittman, *J. Exp. Med.*, 1931, **53**, 471.

The isolation of these organisms strengthens our already expressed belief that the respiratory flora of the Polar Eskimos is very similar to that of persons living in warmer climates without such a great degree of isolation.

6087

The Heat Inactivation of Bacteriophages.*

J. BRONFENBRENNER. (With technical assistance of J. Stokes and W. Moor.)

From the Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis.

In testing the therapeutic value of the lysed cultures of *B. diphtheriae,* it became necessary to destroy the slight amount of toxin present with bacteriophage in some of the filtrates. Since bacteriophage is comparatively resistant to heat it was thought that simple exposure to heat may destroy the toxin without destroying the phage present in the lysates. This, however, we were not able to accomplish. Exposure to heat sufficient to destroy all the toxin caused almost complete destruction of the phage. In a study of the mechanism of inactivation of phages by alcohol we concluded that the effect of alcohol was not due to the direct destruction of the active agent, but to the denaturation of the protein vehicle on which the lytic agent was adsorbed.[1] Suspecting that somewhat similar relation may exist in the inactivation of phages by heat, we attempted 2 series of experiments.

In the first we made use of our earlier finding that the addition of polyvalent cations to the medium containing phage protected it from inactivation by alcohol.[2] We found that the addition of enough $CaCl_2$, for instance, to bring its concentration between 0.002 m and 0.015 m, some degree of protection against heat inactivation may be secured. Optimum concentration of $CaCl_2$ differs with each phage, and it thus becomes necessary to find a suitable concentration for each phage and even for each batch of the same phage, a laborious and time-consuming procedure.

* This work was supported in part from a grant made by the Rockefeller Foundation to Washington University for research in science.

[1] Bronfenbrenner, J., and Korb, C., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 177; *J. Exp. Med.*, 1926, **43**, 71.

[2] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 187.

In later experiments we used another method suggested by Spiro that glycerin, carbohydrates, urea, urethan, etc., prevent denaturation of proteins during coagulation.[3] Recently these and other substances have been used repeatedly for the protection of antibodies and of bacterial antigens against denaturation by heat. In employing a number of these "anticoagulants"[3] we succeeded in some instances in preserving a considerable degree of activity of phages during more or less prolonged exposure of the filtrates to 70°C.

In general the degree of protection varied not only among phages of different valency but among different samples of phages of similar valency. The degree of protection for each phage, in the case of polyvalent phages, is independent of the bacterial substratum used in their preparation. This relation is brought out by comparing the results secured with PC-coli and PC-Shiga phages, or Laudman-coli and Laudman-Shiga phages respectively as illustrated on Table I. In each case one volume of phage was diluted with 3 volumes of broth and 3 volumes of saturated solution of sucrose respectively. The mixtures were titrated before heating and after heating for different periods of time in sealed ampules submerged entirely under water kept at 70°C. The results represent an average of at least 5 independent experiments and are expressed in terms of the smallest fraction of a cc. exhibiting lytic activity. Essentially similar experiments were performed using dextrose, glycerine, sodium salicylate, urea and Bayer 205 in place of saccharose with varying degrees of success.

While prolonged preliminary incubation of bacteria with some of these substances (glycerine or saccharose, for instance), may render them somewhat more resistant to heat, the exposure of bacteria to heat under the conditions exactly duplicating those under

TABLE I.

	Before heating		At 70°C. for 20 min.		30 min.		45 min.	
	Broth	Sucrose	Broth	Sucrose	Broth	Sucrose	Broth	Sucrose
Laudman phage—*B. coli*	10^{-9}	10^{-9}	10^{-4}	10^{-6}	10^{-2}	10^{-5}	0	10^{-4}
" " —*B. Shiga*	10^{-9}	10^{-9}	10^{-3}	10^{-5}	10^{-1}	10^{-5}	0	10^{-4}
P. C. phage—*B. coli*	10^{-9}	10^{-9}	10^{-1}	10^{-6}	0	10^{-5}		
" " —*B. Shiga*	10^{-9}	10^{-9}	10^{-1}	10^{-5}	0	10^{-4}		
Megatherium phage	10^{-9}	10^{-9}	10^{-2}	10^{-6}	0	10^{-4}		
Friedlander phage	10^{-9}	10^{-9}	10^{-5}	10^{-8}	0	10^{-4}		
Diphtheria M 1314 phage	10^{-9}	10^{-9}	10^{-3}	10^{-5}	0	10^{-1}		
" Philippi	10^{-8}	10^{-8}	0	10^{-6}				
Staphylococcus H.	10^{-8}	10^{-8}	0	10^{-3}				
Typhosus phage	10^{-9}	10^{-9}	0	10^{-6}				

[3] Spiro, K., *Zeit. f. Physiol. Chem.*, 1900, **30**, 182; *Hofmeister's Beiträge*, 1904, **4**, 300.

which phages were exposed failed to indicate any protective effect of "anticoagulants" against destruction of bacteria by heat (70°C.). Thus these findings suggest the possibility of employing heat in the presence of saccharose as a means of isolating phages from fecal cultures and from other natural sources instead of employing filtration for the purpose of removing bacteria.

6088

Effects of "K" Medium on the Filterability of Bacteria.

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Kendall employed a culture medium[1] consisting presumably of whole undenatured protein, devoid of all products of digestion which he referred to as peptones. He reported the transformation of ordinary bacteria into primitive forms capable of passing filters presumably impervious to the passage of normal bacteria.[2] Kendall concluded that he was dealing with unusual forms of bacteria, not only on the basis of their filterability, their appearance under the dark-field microscope, and their irregular staining, but also because of his inability to obtain sub-cultures by transplantation of the "filterable" forms to ordinary media, whereas sub-cultures were obtained by similar transplantation to his own, so-called K medium.

We here summarize some of our experiments to acquaint ourselves with this apparently new and important phenomenon.

The appearance of viable and cultivable bacteria in the filtrates of bacterial cultures has been repeatedly recorded. It is generally understood, that the mere passage of cultivable bacteria through filters does not predicate any radical change in their original size. It has been repeatedly shown that under a variety of conditions, normally effective filters may become permeable to bacteria of ordinary and even large size.

Even the first few experiments convinced us that in the case of cultures grown on K medium, the frequency of passage and the numbers of viable units of bacterial protoplasm appearing in the filtrates at each filtration were considerably greater than in the case

[1] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**.

[2] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**.

of cultures grown on ordinary media. Furthermore, the filterability of cultures grown in K medium was the greater the more profuse was the growth attained at the time of filtration. Increased growth could be secured by the addition of carbohydrates or peptone to the K medium and in either case the filterability was increased, thus it is evident that the exclusion of peptones from the medium is certainly not responsible for the increased filterability of Kendall's cultures.

A somewhat quantitative measure of the degree of filterability was secured by using the method of fractional filtration.[3] The filtrates were collected aseptically into test tubes in portions of 10 cc. each, and the series of tubes thus secured were incubated. Appearance of growth in some of the tubes roughly indicated the point at which passage of bacteria began. It was found that growth appeared in the filtrates of cultures grown in K medium much sooner than in corresponding filtrates of broth cultures, apparently due to greater numbers of bacteria passing the filter in the first instance. It must be stated, however, that in all cases subcultures from filtrates of cultures grown either in K medium or in broth were successfully obtained by the transfer to either K medium or to plain broth.

Microscopic examination of cultures developing in K medium either before or after filtration invariably showed marked loss of motility of motile bacteria, with a gradual breaking up of bacterial cytoplasm into beaded forms and free granules, which exhibit very active Brownian movement but not true motility.

To determine the cause of the greater filterability of cultures grown on K medium, we suspended ordinary bacteria (grown on the surface of agar slants) in K medium and in plain broth respectively, and immediately subjected each type of suspension to filtration, allowing no lapse of time for growth and possible transformation of the bacteria to occur. Bacteria suspended in K medium passed the filter more readily than similar suspensions of the same bacteria in saline or plain broth, indicating a marked effect of the K medium upon the filter itself.

Kendall stated[4] that only turbid K medium is suitable for the demonstration of filterable forms, and not the clear K medium. We found that turbid K medium is merely more effective in coating the filter bed. This renders the filters more permeable to bacterial sus-

[3] Bronfenbrenner, J., and Muckenfuss, R., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 371.

[4] Public Lecture at Washington University.

pensions made either in K medium, in saline or in broth, when such suspensions are passed through filters just after passage of sterile K medium.

In addition to the substances in K medium which break down the efficiency of the filters, we thought that K medium might affect the filters because of its high fat content, since as much as 25% by weight of the dry powdered hog intestine base could be extracted by fat solvents. To determine this, we partially clogged filters by the repeated filtration of turbid K medium, and then extracted such filters with ether, alcohol and other fat solvents, and after washing retested their permeability to bacteria. We found that extraction with fat solvents did not restore the efficiency of the filters, whereas if they were cleaned in such a manner as to remove the adsorbed suspended matter, they were again rendered impervious to bacteria.

By inoculating K medium with several phages, Kendall was able to isolate staphylococci from staphylococcus phage. We inoculated sterile K medium, plain broth and blood agar with large inocula of 23 different phages prepared in this laboratory and known to be active. In no case but one was growth observed in the K medium cultures, and in this case growth was also secured in plain broth and on blood agar. The phage from which these cultures were made was known to be contaminated with a diphtheroid, the organism which developed in our cultures. Although transplants were made from the remaining K medium tubes at various intervals, absolutely no sign of growth occurred in such subcultures.

6089

Further Studies on the Mechanism of Transmissible Lysis of Bacteria.*

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Observing carefully the progress of lysis under the microscope we noticed that disappearance of individual bacteria under the influence of phage is usually preceded by more or less marked swelling

* This work was supported in part from a grant made by the Rockefeller Foundation to Washington University for research in science.

of the cells.[1] A more detailed inquiry indicated that the swelling itself was due to imbibition of water which in turn appeared to be the result of intracellular digestion of bacterial cytoplasm,[2] and consequent increase of osmotic pressure within the cells. The actual disappearance of cells from the field was therefore interpreted as due to bursting.[3] We found further that if bacteria are grown on media in which free water was immobilized by the addition to the

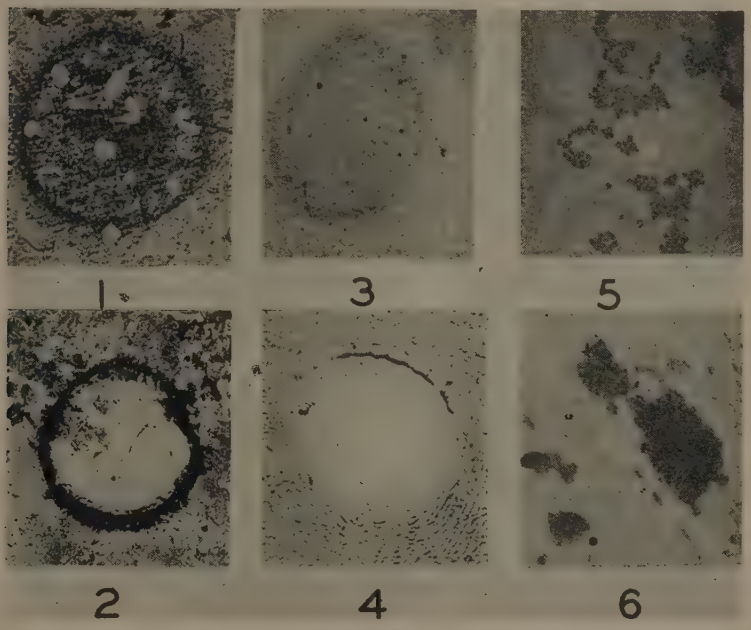

FIG. 1.

1. Stained "Klatsch" preparation showing increased growth of *B. coli* on 4% agar under the drop of bacteriophage. 25×.
2. Same showing lysis of *B. coli* on 1% agar under the drop of bacteriophage. 25×.
3. Direct photograph showing increased growth of *S. aureus* on 50% gelatin under the drop of bacteriophage. 25×.
4. Same showing lysis of *S. aureus* on 1% agar under the drop of bacteriophage. 25×.
5. Stained "Klatsch" preparation showing normal growth of *S. aureus* on 1% agar. 900×, enlarged three diameters.
6. Same showing swollen cells of *S. aureus* on edge of a plaque of lysis on 1% agar. 900×, enlarged three diameters.

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[1] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 635.

[2] Hetler, D. M., and Bronfenbrenner, J., *J. Exp. Med.*, 1928, **48**, 269.

[3] Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, **3**, 562.

medium of hydrophilic colloids, the swelling was prevented and consequently lysis did not occur. On the contrary, in place of a marked fall in the number of bacteria (as would be the case if lysis had occurred), we observed actual increase in the number of bacteria when compared with control cultures grown on the same medium but in the absence of phage.[4] While the above observations were made with Gram-positive and Gram-negative bacteria, the changes were always more constant and more marked in the latter and therefore Gram-negative bacteria were used to illustrate the phenomenon.[5] Recently there appeared a paper in which the authors,[6] working with cultures of Staphylococcus, failed to observe either the swelling or the increased rate of growth of these organisms in the presence of phage. It was imperative to reinvestigate the subject.

The experiments here reported were performed to show that phenomena observed by us during the growth of Gram-negative bacteria in the presence of phage occur also with Gram-positive bacteria.

When either *B. megatherium, B. diphtheriae* or *Staphylococcus aureus* are grown on 4% agar or on 50% gelatine in the presence of phage, they all show increase in the growth rate as compared with that in control cultures grown under the same conditions but with broth added instead of corresponding bacteriophage. Under these conditions lysis does not take place. If, on the other hand, the same organisms are grown on ordinary media, the addition of phage causes swelling and lysis of organisms. The swelling in the case of Gram-positive bacteria is more difficult to record, however, than in the case of Gram-negative bacteria, because the cell membrane of Gram-positive bacteria in general is more permeable, hence the swelling never reaches the same degree as it does in Gram-negative bacteria before the bursting (lysis) takes place.

To obtain the material for demonstration of these phenomena, care must be exercised in securing proper conditions of the surface of the medium which would permit making the "Klatsch" preparations and in selecting suitable proportions between phage and bacterial inoculum so as to limit the lysis to a rate which would permit recording the changes.

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[4] Bronfenbrenner, J., and Hetler, D. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 480.

[5] Bronfenbrenner, J. See monograph on "Filterable Viruses", edited by T. Rivers, (Williams and Wilkins, 1927).

[6] Krueger, A. P., and Northrop, J. H., *J. Gen. Phys.*, 1930, **14**, 223.

6090

Rate of Degeneration in Pure Strains of "Fibroblasts" Possessing Varying Growth Potencies in vitro.

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In a previous paper the author in collaboration with Richardson[1] described variations in the cytolytic changes occurring in undifferentiated and differentiated tissues which were induced to undergo prolonged proliferation in an unchanged medium *in vitro*. The present study was undertaken to extend similar comparative observations upon the rate of cytolysis in pure isolated strains of cells, each possessing different inherent growth rates. Under experimental conditions Parker and Fischer[2] depicted different strains of "Fibroblasts" possessing varying growth potencies, and, for the purpose of these experiments similar tissues were selected and isolated according to the methods described by these investigators. All tissues employed were taken simultaneously from the same embryo of 12 days' incubation and throughout this investigation were explanted under exactly identical conditions from the moment of their isolation *in vitro*. The selected fragments were obtained from the following regions: osteoblasts from the supra-orbital; chondrioblasts from the periostium of the sphenoid (fast growing); and fibroblasts from heart and leg muscles (slow growing).

The first cytolytic response to the accumulations of cyto-toxins in the unchanged medium was morphologically expressed in the slow growing strains after 65-70 hours incubation *in vitro*. Similar examinations, however, of the fast growing strains after the same periods of growth showed them to be more resistant to the factors incurring cytolysis, as degenerative phenomena were not yet apparent. Even when the fast growing osteoblasts and the slow growing muscle-fibroblasts were implanted side by side in the same culture medium, they not only showed the same relative difference in their growth rates, but exhibited the same time variations as when cultivated in their isolated conditions. This experimental evidence suggests that cells are endowed with an inherent intrinsic mechan-

* Rockefeller Foundation Fellow, The Kaiser Wilhelm Institut für Biologie, Gastabteilung Dr. A. Fischer aus Kopenhagen.

[1] Horning, E. S., and Richardson, K. C., *Aust. J. Exp. Biol. and Med. Science*, 1929, **6**, 229.

[2] Parker, P. C., and Fischer, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 580.

ism and, moreover, that cell-behavior *in vitro* is not solely determined by the extrinsic physiological conditions of the culture medium.

After 65-80 hours' growth, a very interesting reaction to the toxicity of the unchanged medium was first observed in the slow growing cultures. Following general hypertrophy of the protoplasmic inclusions, many of the cells, especially those on the peripheral margins of the areas of new growth, underwent an unequal division of the cytoplasm. The nucleus played no apparent rôle and remained in the resting phase during this abnormal process. As the 'daughter portion' contained no nuclear material, and was crowded with hypertrophied inclusions, it might be assumed that this is an effort on the part of the degenerating cell to restore the nucleo-cytoplasmic ratio. When the apparent karyoprotoplasmic relations have been restored the cell survives while the 'daughter portion' disintegrates within the culture medium. Evidence of such a phenomenon does not become apparent in the fast growing strains until after 90-95 hours' growth. As far as could be estimated the phenomenon described above is more or less specific to the slow growing strains, but has, nevertheless, occasionally been detected in the osteoblast cultures after 100 hours incubation.

Other evidences of cytolysis such as chondriolysis, vacuolation, and lipoid formation which have previously been described in detail[3] were prevalent degenerative features common to all strains, but appeared first in the cells of the heart and muscle fibroblasts.

As cytolysis proceeds after 90 hours' incubation the cells of the slow growing fibroblasts further respond to the exhaustion of the culture medium by a marked hypertrophy and intense pseudopodial outgrowths and so much alter their contours that a differentiation is immediately suggested. The cells at this period presented a totally different morphological picture, having completely lost their spindle-shaped fibroblast-like characters. In order to observe the mechanism underlying this apparent differentiation, pure cultures of heart and muscle fibroblasts of 48 hours' growth were cut into equal portions and after fresh media had been added were sealed to a concavity slide upon which a film of H_2O_2 had been previously made. An equal number of remaining cultures were employed as controls. Examination after 90 hours in an unchanged medium revealed a striking difference between the experimental and control explants. The former retained to a large degree their typical spindle-like structure, while the controls displayed marked alterations in

[3] Horning, E. S., and Richardson, K. C., *Aust. J. Exp. Biol. and Med. Science*, 1929, **6**, 229.

their morphology. This experiment suggests that the "differentiation" was purely a superficial process, and was probably an effort on the part of the cell to increase its surface area owing to lack of oxygen, through cultivation in an unchanged medium. This might possibly explain the results of earlier workers who held that fully differentiated tissue during growth *in vitro* reverts back to an indifferent cell-type.[4]

Hyperchromasy which is generally followed by chromidiosis was frequently observed in the slower growing "fibroblasts" after 100 hours growth *in vitro*, and was very rarely detected in the osteoblast and chondrioblast cultures. These observations support the contentions of previous authors who regard it as representing a pathological rather than a normal state. This phenomenon might be interpreted in view of the hypothesis of Popoff, who contends that this is a function wherein the mass relation between the nucleus and the cytosome might be restored, if for any reason the amount of chromatin relating to the cell protoplasm has increased. Comparative observations of a large series of cultures showed in all cases that the fast growing strains are more resistant to pathological conditions than the slow growing tissues. The final evidence of cytolysis is manifested in the heart and muscle fibroblasts after 10 days' incubation in the unchanged medium. Fusion of fat globules occurs and tension forces within the cell interior are responsible for the extremely bizarre morphology of the cultures. Chromatolysis which becomes apparent on the ninth or tenth day, is invariably followed by plastin hypertrophy of the nucleolus. This association of nucleolar change with absorption of chromatin material is significant, as it suggests the possibility that this structure is built up of waste nuclear material. As soon as the nucleus collapses cell death occurs, and in order to verify this fact the cultures were transferred to fresh media and in all cases they failed to exhibit signs of renewed growth; while disintegration of the faster growing osteoblasts and chondrioblasts did not set in until the fourteenth or fifteenth day *in vitro*.

In conclusion it might be assumed that tissue cells which exhibit similar morphological characteristics and behavior under normal conditions *in vitro*, differ however in their reactions to similar induced pathological conditions, as the rate of cytolysis was in all cases found to be dependent upon the inherent growth energy of the given strain.

I wish to express my indebtedness to Dr. Albert Fischer for his interest and suggestions during this investigation.

[4] Champy, C., *Bibliog. Anat.*, 1913, **33**, 184.

New York Meeting.

New York Academy of Medicine, April 20, 1932.

6091

Quantitative Difference in a Rabbit-Ovulating Dose of Prolan and Anterior Pituitary Extract.

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It was previously reported[1] possible to obtain follicular development and corpora lutea formation in immature female rats with a dose of pregnancy urine which was inadequate to induce ovulation in an adult female rabbit in heat. Friedman[2] has recently shown that the ovulating dose of Prolan (from pregnancy urine) is equal to approximately one rat unit per kilogram of body weight of the rabbit. This report will attempt to show that this quantitative relationship does not hold when using an anterior pituitary extract.

The extract used in these experiments was the water soluble fraction of a pyridine extract of sheep anterior pituitary, capable of producing follicles and a few corpora lutea in immature rat ovaries.[3] It was administered in 1 cc. of distilled water intravenously into adult rabbits in heat and 15-20 hours later the ovaries were examined grossly for ovulation. The immature rats were injected subcutaneously (0.25 cm. doses) twice daily for 5 days and killed on the beginning of the 6th day. Sixteen rabbits and 9 rats were used in these experiments.

In the first group, doses of the extract from .025 gm. equivalent of dry powdered gland to .0025 gm. gave positive ovulation. Two

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* National Research Fellow in the Biological Sciences.

† Aided in part by a grant from the National Research Council, Committee on Problems of Sex, administered by Dr. P. E. Smith.

The sheep pituitaries were kindly furnished by Parke Davis Co.

[1] Leonard, S. L., *Am. J. Physiol.*, 1931, **98**, 406.

[2] Friedman, M., *J. Exp. Pharm. and Therap.*, 1932, in press.

[3] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1931, **97**, 291.

months later the M.O.D. (minimal ovulating dose) was found to be .005 gm., indicating a slight deterioration of the extract. However, if amounts of the same extract equal to .025 or .0125 gm. of anterior lobe were given to sexually immature female rats, no apparent morphological or physiological changes were produced in the ovaries after 5 days' treatment (Table I). These amounts are

TABLE I.
Failure of a Rabbit Ovulating Dose of Anterior Pituitary Extract to Stimulate Ovaries of 22-day-old Rats.

Rat	Total Dose S.A.P. 1	No. Days Injected	Vagina at Autopsy	Wt. of Uterus and Vagina	Wt. of Ovaries
				gm.	gm.
GH5971 a	Control	—	Closed	.0535	.0120
b	.025 gm.	5	"	.0625	.0105
c	.025 gm.	5	"	.0835	.0123
d	.0125 gm.	5	"	.0610	.0095
e	.0125 gm.	5	"	.0770	.0110

several times the M.O.D. for rabbits, which emphasizes the difference from Prolan. The experiment was repeated using a different batch of hormone prepared in the same manner and the results were comparatively identical, although the M.O.D in this case was between .005 gm. and .0075 gm. Again, several times the M.O.D. of the extract was without effect on the immature rats.

It should be pointed out that the rabbits must be isolated for 15-20 days or more before they are used again. In another series of 4 rabbits, it was found that the M.O.D. was not sufficient to induce ovulation in an adult rabbit which had been stimulated 10 days previously, even though grossly the ovaries contained apparently normal ripe follicles. A larger dose, however, will induce ovulation under such conditions. Therefore, a standard rabbit should be adopted in determining the M.O.D. of these extracts.

Conclusions. 1. The ovulating dose of an anterior pituitary extract for rabbits is very much less than that of Prolan when the potency of these 2 substances is standardized on immature female rats. 2. This difference in quantitative activity of Prolan and anterior lobe extract suggests a physiological difference between the 2 substances.

6092

Effect of X-Ray on Poliomyelitis Virus in vivo and in vitro.*

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The use of Roentgen rays as an adjuvant in the treatment of human poliomyelitis has been widely recommended by a number of clinicians[1] while others[2] have expressed their doubt regarding the value of this form of therapy. Experimentally, the problem has apparently never been approached save for some earlier work of Amoss, Taylor and Witherbee.[3] These experiments, although originally undertaken for a different purpose, served to demonstrate that monkeys subjected to large doses of X-ray developed a more fulminant type of infection than did the controls.

In view of the conflicting clinical reports and in the absence of pertinent experimental data, it appeared worthwhile to investigate whether poliomyelitic monkeys upon exposure to very small doses of X-ray, such as are used successfully in a variety of acute inflammatory conditions, would not benefit from such treatment. There seemed to be at least a possibility that the quicker regression of paralysis alleged to have happened occasionally in human cases after early treatment with X-ray, might have been due to an acceleration in resorption of the perineuronal edema, mechanical pressure of which is generally conceded to exert a deleterious effect on the ganglion cells long before actual destruction has resulted in permanent damage.[4] At the same time it was interesting to determine whether raying of virus *in vitro* with much larger doses would affect the virulence of the infectious agent.

The experimental data submitted in this paper deal with observations on a total of 12 rhesus monkeys, exclusive of controls, which are distributed over the following 3 groups:

I. Effect of X-ray after onset of paralysis. Seven monkeys

* Under a grant from the International Committee for the study of infantile paralysis whose work is being financed by Jeremiah Milbank.

[1] Bordier, H., *Rev. gén. clin. thérap.*, 1931, **45**, 465. Delherm, L., *J. Radiol. et Electrol.*, 1931, **15**, 321.

[2] Marinesco, G., Manicatide, M., and Draganesco, St., *Ann. Inst. Past.*, 1929, **43**, 223.

[3] Amoss, H. L., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, **24**, 115.

[4] Aycock, W. L., and Amoss, H. L., *Bull. Johns Hopkins Hosp.*, 1923, **34**, 361.

with either incipient or almost completely developed paralysis were subjected to one or repeated X-ray treatments. The rays were directed on the areas corresponding to the cervical and lumbar enlargements of the spinal cord. The factors used were 200 KV, 8MA, 1/2 mm. Cu+1A1 filter, TSD 57 cm., fields 6x10 to 6x25 cm. 100 r or approximately 1/6 of an erythema dose was administered for 3 minutes during each treatment. Of the 7 rayed animals 2 were found dead the following morning and 2 others died on the second day after treatment; the remaining animals, with the exception of one, dying at later periods of time. In 6 instances paralysis progressed as usual and histological examination revealed lesions in the cord of almost greater intensity than commonly observed in controls. The one surviving monkey appeared to improve rapidly during the course of further treatments. However, it is more likely that the stated improvement should be attributed to chance rather than to the effect of the X-ray.

II. Effect of X-ray during incubation period. Two monkeys were exposed to X-ray (dosage same as above) during the incubation period of the disease, the first treatment being given on the day of infection over the local area of intracerebral inoculation. This was followed by another treatment, 3 days later, applied to the cervical and lumbar cord. Both monkeys developed typical poliomyelitis within 6 days, leading eventually to complete paralysis and prostration, with no significant difference in the course of the disease from that of an accompanying control animal. It is of interest to note in passing, that after the first exposure to X-ray these monkeys, both of which were immature male animals, showed a peculiar testicular reaction characterized by sudden swelling of the organ and a tendency for descent. This may possibly be interpreted as evidence of action on the pituitary gland.

III. Effect of X-ray on virus in vitro. Three monkeys, in separate tests, were inoculated intracerebrally with 1 cc. of a 10% suspension of poliomyelitic cord, which had previously been exposed to large doses of X-ray. (200 KV, 8MA, 2 cm. wood filter, 50 cm. target distance, 38 minutes.) Two of these monkeys came down with poliomyelitis after an incubation period of 12 and 13 days, respectively, while the third animal developed the disease in a typical manner on the sixth day after inoculation. In all 3 monkeys paralysis progressed to complete prostration. Although the incubation period in 2 of these monkeys was somewhat prolonged over the average seen in controls, it would seem that the variation is still within the limits of spontaneous fluctuation of virulence of the virus.

In concluding it seems safe to assert that under the experimental conditions employed in our work, exposure to X-ray failed to destroy or markedly attenuate the virus *in vitro*. The observations on monkeys suffering from the experimental infection and treated either during the incubation period or with manifest paralytic symptoms furnish no evidence suggesting any benefit from the Roentgentherapy as used. It is difficult to properly evaluate these findings in their bearing upon the usefulness of Roentgentherapy during the course of the human disease. It should be remembered that the infection in the monkey is much more severe and recovery is exceedingly rare after complete paralysis has developed. In the human, on the other hand, paralysis shows a tendency for spontaneous regression in the majority of the cases. The argument therefore is ambiguous, as it may be held in favor as well as adverse to any possible value of X-ray in the treatment of human poliomyelitis.

6093

Experimental Enhancement of Malignancy in the Brown-Pearce Rabbit Tumor.

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The Brown-Pearce rabbit tumor is a transplantable malignant epithelioma carried in this laboratory for more than 100 generations by intratesticular inoculation. It has been used extensively for the study of animal constitution and it has been found possible to alter the susceptibility or resistance of animals to this tumor by various surgical and environmental procedures. About a year and a half ago attempts were made to alter the resistance or susceptibility of animals to inoculation with this tumor by the use of material derived from the tumor itself. The essential feature concerned the preparation of the material for use in conjunction with a regular tumor inoculation.

The first attempt resulted in a marked enhancement.[1] A rabbit which had died 5 to 10 hours previously of the Brown-Pearce tumor was placed for 2 weeks in the ice-box (26-32°F.) At the termination of this period, a normal saline emulsion of the primary tumor

[1] With reference to enhancing extracts from various tissues and from other tumors see: Chambers, H., and Scott, G. M., *Brit. J. Exp. Path.*, 1924, **5**, 1.

(testicle) was made and 0.3 cc. inoculated into the right testicles of each of a group of normal young adult rabbits. Two weeks later 0.3 cc. of a normal saline emulsion of fresh Brown-Pearce tumor was inoculated into the left testicles of each of the same group of rabbits and also into each of a control group not previously inoculated.

This first experiment was repeated 7 times, and the only change in the experimental procedure was to remove the tumor from the rabbit, imbed it in paraffin, and preserve the imbedded tumor in the ice-box before using. All experiments were terminated at the end of a 2 months' period after the last inoculation and the results in every experiment confirmed the original observations that a significant enhancement of malignancy had occurred. Of the 43 animals inoculated 100% grew primary tumors as against 68% among the 119 animals in the control series; the size of the primary tumors at autopsy was 22 cc. as against 10 cc. in the controls; the mean longevity was 44 days against 56 days for the controls; the mean mortality based upon the number of deaths, probable deaths, and recoveries was 95% as against 57% in the controls; the mean number of metastatic foci was 18 as against 8 in the controls; the incidence of metastases was 100% as against 65% in the controls; and the total tumor growth per animal, including both primary and metastatic, was 146 cc. as against 56 cc. in the controls.

This enhancement of malignancy has been and is being investigated in a large number of experiments, most of which are still in progress. The procedure has been to change each variable in the original equation quantitatively and qualitatively in new experiments. Concerning the source of material suitable for preservation, it has been found possible to use fresh, semi-necrotic, or necrotic tumor; to obtain tumor from a recently inoculated, moribund, or dead animal; to use the primary growth or omental or retroperitoneal metastases. The inoculation of fresh tumor, or fresh or preserved normal testicle 2 weeks before tumor inoculation has not resulted in enhanced tumor growth.

Concerning methods suitable for preservation, it has been found possible to keep the rabbit for 2 months in the ice-box (26-32°F.) instead of the original 2 weeks; to preserve for 10 days only, but this often resulted in a local growth after inoculation of the opposite testicle. The preserved material can be inoculated into either testicle, into the skin, subcutaneous tissue, or muscle. Enhancement followed inoculation of preserved material both at the same time and 2 weeks later than the fresh tumor. A single series of animals

was inoculated at 60-day intervals with preserved material alone, and after 5 months all came down with primary tumors at the site of the last injection. This was followed by widespread tumor metastases, and death in all of the rabbits. Inasmuch as the preserved material which was used for the last injection had been refrigerated for 10 days instead of the usual 2 weeks, the possible presence of living cells capable of growth could not be ignored.

A definite enhancement, though somewhat lessened, has followed filtration of the preserved tumor material through "V" Berkefeld filters. The use of desiccated preserved material also results in a definite enhancement.

Animals of various age, sex, and breeds that have been tested so far have been suitable for inoculation with preserved material. A series of thoroughly tested and retested immune animals were inoculated with preserved material followed in 2 weeks by tumor inoculation. Thirty-three per cent of these immune rabbits grew malignant tumors; the others remained negative. Metastatic growth does not ordinarily occur from intracutaneous inoculation, but widespread metastases and death have resulted from the use of preserved material before skin inoculation.

6094

Some Vital Staining Reactions Bearing upon the Homology of Spermatocyte Dictyosomes.*

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In male germ cells the well-known dictyosomes and their derivatives, the acroblasts, are vigorously blackened by silver or osmium impregnation methods. Therefore they have been termed "Golgi bodies" and are accepted as complete homologues of the Golgi-apparatus of mammalian nerve and gland cells. The Golgi-apparatus is believed to be concerned with the function of secretion. An example frequently cited is that the acrosome of the animal sperm is secreted by the dictyosome complex, involving the tacit assumption that this complex is the homologue of the Golgi-apparatus. Recently this

* This work was made possible through a grant from the Educational Fund, King Solomon Lodge No. 279, F. & A. M., New York.

homology has been questioned. Parat[1] and his coworkers, for example, consider the dictyosomes to be chondriosomes of large and active type, partly for the reason that the dictyosomes (Parat's "lepidosomes") of the living cell stain with Janus green nearly as vigorously as the chondriosomes. Both the staining reaction and the implied homology have been doubted.[2, 3] It therefore seemed desirable to reinvestigate the problem.

The present account presents some results of intra-vitam staining of metamorphosing male germ cells of various insects. The species used were 2 Orthoptera of the family Acrididae, *Rhomaleum micropterum* and *Melanoplus femur-rubrum,* and 3 gryllid Orthoptera, *Œcanthus nigricornis, Nemobius fasciatus* and *Gryllus assimilis* var. *luctuosus.* In the experiments with Janus green an hemipter, *Euschistus euschistoides,* was also used. Most of the work was done at the Marine Biological Laboratory, Woods Hole, Mass. The method used has been described previously.[4]

It is generally agreed that the mammalian Golgi-apparatus is not observed intra-vitam.[5, 6, 7] Several recent workers, however, have been able to see the dictyosomes clearly in unstained living spermatocytes. To the cases cited by Bowen[7] I have added the gryllid Orthoptera[4] and the insects listed above. In each of these the chondriosomes and the chromophilic part of the dictyosomes have almost the same high refringence and are easily identified in the unstained cell. Compared with the non-refringent Golgi-apparatus of the somatic cell, the dictyosome presents a physical difference which probably finds its basis in a dissimilar chemical structure. This assumption receives additional support from the vital or semi-vital staining reactions to be described.

Cowdry[8] has established that Janus green staining is the surest method for the demonstration of mitochondria (chondriosomes). It is agreed that in the mammalian somatic cell the Golgi-apparatus ordinarily is not stained with this vital dye (Gatenby,[9] Bowen,[7] Beams[5, 6]). However, when Janus green is applied to fresh smears

[1] Parat, M., *Arch. d'Anat. mic.,* 1928, **24**, 73.

[2] Hirschler, J., *Z. Zellf. mikr. Anat.,* 1928, **7**, 62.

[3] Gatenby, J. B., *Proc. Roy. Soc. London,* 1929, **104**, 302.

[4] Johnson, H. H., *Z. f. wiss. Zool.,* 1931, **140**, 115.

[5] Beams, H. W., *Anat. Rec.,* 1930, **45**, 137.

[6] Beams, H. W., *Anat. Rec.,* 1931, **49**, 309.

[7] Bowen, R. H., *Anat. Rec.,* 1928, **38**, 293.

[8] Cowdry, E. V., *Contrib. to Embryol.* (Carnegie Inst.) Washington, 1918, **8**, 39.

[9] Gatenby, J. B., Lee's Microtomist's Vade-mecum, 8th ed., P. Blakiston's Son & Co., Philadelphia, 1924.

of insect spermatocytes, vigorous coloration of chondriosomes and also the chromophilic part of each dictyosome results. In the spermatids the same is true; both the nebenkern and the acroblast complex are colored. This reaction has been obtained repeatedly in all of the insects studied, and a few tests gave the same result in Hemiptera of the family Reduviidae. These color reactions were obtained both with Gruebler's Janus green and with diazine green of National Aniline Co. Other mitochondrial dyes were used also. Methyl violet B (dahlia violet), while it kills the cell quickly, colors vigorously both chondriosomes and the crescentic, chromophilic rims of the dictyosomes.

In preliminary experiments with benzidine dyes, which ordinarily are not considered to be mitochondrial stains (see Gatenby[9]), similar results were obtained. The dyes used were trypan blue, trypan red, and pyrrol (isamine) blue. They tint both the chondriosomes and the chromophilic portions of dictyosomes to the exclusion of other parts of the cell, except the cytoplasmic chromatoid bodies (not the chromosomes). The color is rather delicate; the stains are not so vigorous as Janus green.

I have also used brilliant cresyl blue, which stains the chromophilic rims of the dictyosomes (and acroblasts) selectively, although prolonged application colors the chondriosomes lightly. Both are colored prior to the appearance of the well-known cresyl blue vacuoles, which I believe to be degeneration vacuoles. After slight application, the dictyosomes and cytoplasmic chromatoid bodies alone are colored. Fauré-Fremiet[10] and Karpova[11] were not able to stain dictyosomes with brilliant cresyl blue, but Avel[12] obtained a color reaction on the dictyosomes of the snail with it. The bodies of Perroncito in snail spermatids, likewise colorable with this dye, have been identified by Tuzet[13] as fragments of the acroblast remnant. Apparently the converse result is obtained with vital neutral fuchsine; my preliminary tests indicate that with it the chondriosomes alone are stained.

In a former work[4] I adduced evidence that chondriosomes and the chromophilic rim of the dictyosomes are chemically allied, namely: 1, both react positively to Sudan III; 2, they are preserved, stained or impregnated (as the case may be) in the same substances, differing only in degree; 3, both are stained vitally with Janus green.

[10] Fauré-Fremiet, E., *Arch. d'Anat. mic.*, 1910, **11**, 457.
[11] Karpova, L., *Z. f. Zellf., u. mikr. Anat.*, 1925, **5**.
[12] Avel, M., *C. R. Soc. Biol., Paris*, 1925, **93**, 161.
[13] Tuzet, O., *Arch. de Zool. exp. et gén.*, 1930, **70**, 62.

The present paper confirms and extends the third point, and adds a fourth, viz., confirmatory results obtained with benzidine dyes. If the dictyosome of the male germ cell is to be considered a homologue of the somatic Golgi-apparatus, the former must be composed in part of, or perhaps be invested with, a substance not present in or on the Golgi-apparatus. This substance, apparently much like chondriosomal substance, is refringent (probably lipoidal) in nature, causing the dictyosome to be visible even in the absence of stain. The material is perhaps present in widely divergent animal types. It is especially apparent in dictyosomes of gastropod Mollusca, well known for their marked refringence and susceptibility to vital staining, as demonstrated by Gatenby,[14] Avel,[12] and Karpova.[11] In prosobranchs, however, Tuzet[18] failed to stain the dictyosomes with Janus green, but she attaches little importance to the Janus green reaction, since she had formerly obtained a positive result in *Tubularia*.

In common with the post-vital reactions,[4] the results obtained with methyl violet, Janus green and benzidine dyes offer no obstacle to Parat's claim that the chromophilic part of the dictyosome is merely an hypertrophied chondriosome. That such conclusion is perhaps premature is indicated by the specific results obtained with brilliant cresyl blue (selective for dictyosomes) and with neutral fuchsine (selective for chondriosomes), but the value of these reactions for purposes of analysis may be, in some measure, open to question.

This study indicates that the complete homology of the dictyosome-acroblast complex of insect spermatogenesis to the mammalian somatic Golgi-apparatus remains to be established beyond a doubt, while the same might be said with equal justification of its affinities with the chondriosomes. Meanwhile the possibility remains that the complex in question is unique and peculiar to germ cells.

[14] Gatenby, J. B., *Quart. J. Mic. Sci.*, 1920, **64**.

6095

Production of Goiter and Exophthalmos in Rabbits by Administration of Cyanide.

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We have shown that several of the organic cyanides[1] when injected subcutaneously into rabbits daily for 3 or more weeks produce thyroid hyperplasia. Methyl cyanide produces the greatest thyroid reactions, and young rabbits (3 to 4 months) are more reactive than adults to the same doses per kg.

Chronic bilateral exophthalmos has occurred in a significant number of prepubertal rabbits after methyl cyanide has been given for varying lengths of time. Exophthalmos has developed as early as the 20th day after beginning daily injections of 0.1 cc. of methyl cyanide in 3 months old rabbits. Of the 2 breeds used (Dutch and Belgian) we have observed it most frequently in young Dutch rabbits. It has not been detected in adults (6 months and over) in either strain. Representative instances are given in Table I.

So far we have observed only moderate degrees of exophthalmos. It occurs earliest in those rabbits which develop thyroid hyperplasia the quickest and appears to be proportional to the degree of thyroid hyperplasia. Rabbits that have failed to develop thyroid hyperplasia, or rabbits in which the hyperplasia has been slight have not developed exophthalmos. When exophthalmos is develop-

TABLE I.

No. Exp.	Sex	Age	Breed	Weight	Daily Dosage CH_3CN	No. days Before Exoph. Detected	Condition of Thyroid	Remarks
		(mo.)		(gm.)	(cc.)			
175	M	4	Dutch	1184	0.1	39	+++	
"	M	4	"	1341	"	60	+++	
"	F	4	"	1410	"	—	—	No exoph. 63rd day
187	M	3	Belgian	1795	"	—	+±	No exoph. 29th day
"	M	3	Dutch	1491	"	20	++	
"	M	3	"	1564	"	28	+±	
"	F	3	Belgian	1862	"	—	+	No exoph. 29th day
"	M	3	Dutch	1410	"	22	++	
"	M	3	"	1767	"	—	+	No exoph. 29th day
160	M	5	Belgian	1911	0.15	—	—+	No exoph. 25th day
"	M	5	"	1908	"	—	+±	No exoph. 25th day

* Fellow of the Rockefeller Foundation.

† Aided by a grant from the Ella Sachs Plotz Foundation.

1 Marine, D., Baumann, E. J., Spence, A. W., and Cipra, Anna, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, in press.

ing the rabbits become nervous and fidgety. This is frequently associated with soft feces and an increase in the volume of urine.

No attempt has been made to determine what rôle the hormones of the posterior pituitary and chromaffin tissue may play in the production and maintenance of the exophthalmos. There is, however, some hypertrophy and great hyperemia of the medulla of the suprarenals in these animals.

6096

Survival Period of Bilaterally Adrenalectomized Rats.

ROBERT GAUNT.* (Introduced by W. W. Swingle.)

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Wide discrepancies exist in the literature as to the life-span of adrenalectomized rats. Most of the work between 1900 and 1930 indicated that approximately 50% of these mammals survived adrenal ablation for a month or indefinitely. This survival was attributed to the presence of accessory cortical tissue. Recently, however, Pencharz, Olmsted and Giragossintz[1, 2] reported that the rat is no exception to the rule that adrenalectomy is fatal in mammals. Freed, Brownfield and Evans[3] stated definitely that adrenalectomy was uniformly fatal in rats if one-quarter inch of the pedicle was removed along with the gland. Kutz[4] stated that out of 57 animals of his strain operated at 4 weeks age, 56 were dead by the tenth day. While these results were being reported other workers have been reporting a high percentage survival. The present study was undertaken in an attempt to give some explanation to this chaos of evidence.

A series of 156 adrenalectomies were done to investigate the effects of different types of operation in animals of different ages and different strains. Rats from 35 days to 10 months age were used from 5 different colonies. To demonstrate that the animals

* This study was suggested by Professor W. W. Swingle and his advice and assistance have been generously given.

[1] Pencharz, R. I., Olmsted, J. M. D., and Giragossintz, G., *Science*, 1930, **78**, 175.

[2] Pencharz, R. I., Olmsted, J. M. D., and Giragossintz, G., *Phys. Zool.*, 1931, **4**, 501.

[3] Freed, S. C., Brownfield, B., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 1.

[4] Kutz, R. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 91.

were really dying of adrenal insufficiency a series from all colonies were revived from their terminal coma with the cortical hormone, kindly supplied by Drs. W. W. Swingle and J. J. Pfiffner. Also to check the effect of surgical trauma a series of control operations were done. Rigid asepsis was maintained in the operations. A balanced diet containing all necessary food factors was used. No animal was used unless in apparent perfect condition of health.

Results. The results indicate that different strains or colonies of rats obtained from various sources over the country differ remarkably in their post-adrenalectomy survival; and this difference is of sufficient magnitude to account for many of the previous divergent reports. Animals from 4 of the 5 colonies gave essentially similar results, an almost uniform fatality, essentially like that reported by Pencharz, Olmsted and Giragossintz. Rats from these colonies nearly all died by or before the fifteenth day with a survival for a month or longer of but 5%. The average life-span of this group was 7 days; and 60% of the deaths fell between 4½ and 10 days.

The fifth or "T-Colony" showed an entirely different picture. To date it has been possible to get only 26 of these animals for operation, but of that number exactly half or 13 of them are still surviving at more than 30 days after adrenalectomy, and most are apparently in excellent condition. Of the 13 which have died, the average life-span was 14.2 days, twice that of the other colonies. The survival period varied between 5 and 27 days. These results compare favorably with many earlier reports on long survival after adrenalectomy. It should be emphasized that exactly the same operative technique and post-operative care was employed for all animals.

The operation used by Freed *et al.*, Pencharz *et al.*, in which the surrounding fat, connective tissue and pedicle were removed along with the adrenals proper was employed in approximately half of the cases of all colonies. No significant difference has been demonstrated in the results following this operation and that involving merely removal of the adrenals.

Young animals are apparently somewhat more susceptible to adrenalectomy than older ones. Sex makes no apparent difference. Accessory cortical tissue has been observed in only a few animals. It can generally be found in those living over a month in the few cases in which necropsy has been performed (most of these animals are still living). Rarely, however, is it seen, even after histological examination of any suspicious-looking tissue, in animals living less than one month. No other feasible explanation exists, however, to

explain the extended survival of part of the animals than to presume the presence of minute amounts of accessory tissue; because rats kept on cortical extract show a quick sensitivity either to the presence or absence of that hormone.

6097

A New Roentgenologic Technic in the Study of Phonetics.

LEON J. MENVILLE AND J. N. ANÉ.

From the Department of Medicine, Tulane University, New Orleans.

Numerous theories have been advanced and investigations made to account for differences in voice quality based upon a study of speech sound after it leaves the mouth. Such studies have been in many instances unreliable because formerly it was almost impossible to make an accurate study of the intra-oral, pharyngeal, and laryngeal mechanisms, which are among the important structures used to produce speech and singing.

About 20 years ago it was appreciated that the X-ray, by outlining the forms and sizes of speech and singing cavities, could be the means of uncovering many of the hidden secrets of phonation. Myers[1] demonstrated a certain technic by which these structures were visualized on an X-ray plate. Later, Stephen Jones[2] devised the chain technic, which consisted of the passing of a small chain through the nostril of a subject who was instructed to swallow the loose end. An X-ray plate was then made to show the position of the chain in relation to the soft tissues. Russell[3] used a fine thread which apparently was impregnated with some substance opaque to the X-ray. The subject was made to swallow the loose end of the thread, which was supposed to remain by capillary attraction on the middle portion of the tongue. We are unable to find mention by any of the numerous investigators that the palate was satisfactorily outlined by any marker except the normal bone as shown on the X-ray film.

It must be appreciated that a mechanical means employed in outlining the forms and sizes of speech and singing cavities, such as the chain technic, gold foil, or the thread technic, could interfere

[1] Russell, G. Oscar, Speech and Voice, The Macmillan Co., New York, 1931, 7.
[2] Russell, G. Oscar, Speech and Voice, The Macmillan Co., New York, 1931, 7.
[3] Russell, G. Oscar, Speech and Voice, The Macmillan Co., New York, 1931, 10.

with the normal functioning of the anatomic structures which produce speech. In addition, it is difficult for a small thread to remain in constant alignment on the middle of the tongue, and also on the middle of the hard and soft palate.

In the course of Andrade's[4] experimental investigation on the articulation of certain sounds in the speech of Mayan Indians, we were privileged to conduct the roentgenologic phase of these experiments, making use of what we believe is a new roentgenographic technic. It was suggested by one of us that, instead of using such mechanical markers as were employed by earlier investigators, a mixture of gum acacia and barium sulphate be painted with a camel's hair brush on the midportion of the tongue from base to tip, also that the soft and hard palate be bisected with a coating of the same mixture. While this demonstrated roentgenologically the midsection of the tongue and palate, after a while the saliva caused the mixture to spread. It was then suggested that a preparation of glucose and bismuth subcarbonate be used. This proved to be an ideal marker, as it cast a dense shadow when roentgenographed and the movements of the tongue and the saliva did not efface the preparation.

Some of the more representative X-ray films in which this technic was used are here demonstrated. The midportion of the tongue is clearly shown and its relation to the palate, which is also marked, is demonstrated. The relation of the tongue to the hard palate is considered of great importance in the study of the articulation of all vowels.

In our roentgenographic examination, we used a soft-tissue technic which makes possible the visualization of the epiglottis, the larynx with its vocal cords, and the position of the epiglottis in relation to the base of the tongue, which is considered of importance in phonologic studies.

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[4] Andrade, Manuel J., of the Carnegie Institution of Washington. The Chichen-Itza Project. Middle American Archaeological Research. Verbal communication, 1931.

6098

Oxygen Absorption Through Skin. Effect Upon the Vascular Reaction to Stasis and to Histamine.

SAMUEL GOLDSCHMIDT AND BARTGIS MC GLONE.

From the Department of Physiology, University of Pennsylvania.

The cyanosis which develops in an arm with the circulation totally or partially occluded, may fail to appear when the part is surrounded by an atmosphere of oxygen. The skin of the forearm is usually maintained free of cyanosis under these conditions, while the fingers especially and the hand are quite refractory. This investigation, suggested by the work of Shaw and his coworkers,[1] is quite consistent with their finding that oxygen is absorbed by the skin. However, contrary to the interpretation which they place upon their work, we must conclude that, under the conditions of our experiments, the oxygen finds its way into the blood of those vessels which give color to the skin.

The reactive hyperemia, which invariably appears upon the release of a circulatory occlusion of an arm in air or nitrogen, is absent in an arm which has been in an atmosphere of oxygen during the period of circulatory arrest, except in those parts which were cyanosed or "dusky" before the release, *i. e.*, the fingers and hand. The oxygen, therefore, would seem to prevent the dilatation of the skin vessels which prevails in an arm with arrested circulation.

The failure to obtain a reactive hyperemia under these conditions was examined in the light of the hypothesis of Lewis,[2] which ascribes the control of the small vessels of the skin to an "H" substance, a normal metabolite, the identity of which with histamine Lewis believes to be established by a wealth of convincing evidence.

The findings here recorded would seem to indicate that either histamine is not produced or it is destroyed under prevailing conditions. The idea as developed by Lewis precludes the first possibility, since if one assumes that dilatation of the small vessels of the skin under a variety of physiological conditions is due to the "H" substance, one must of necessity, as indeed Lewis does, postulate that the substance is a normal skin constituent, present in but small amounts normally, but piling up when the circulation is arrested.

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[1] Shaw, L. A., Messer, A. C., and Weiss, Soma, *Am. J. Phys.*, 1929, **90**, 107. Shaw, L. A., and Messer, A. C., *Am. J. Phys.*, 1930, **95**, 13; 1931, **98**, 93.

[2] Lewis, Thomas, "The Blood Vessels of the Human Skin and Their Responses." London, 1927.

As to the second possibility, a destruction of the histamine by the high oxygen tension, it might be expected, if this were the explanation of our findings, that there would be a diminution or failure of the histamine reaction to develop when histamine is introduced intradermally into an arm in oxygen. On the contrary, the reaction is obtained even in very high dilutions. Indeed, under these conditions one observes a well-defined flare, a fact also incompatible with Lewis' belief that this part of the "triple reaction" of histamine is exclusively a reflex arteriolar dilatation.

6099

Effect of Salt Concentration on the Colorimetric Phosphorus Determination.

JOHN MUNSELL. (Introduced by S. Morgulis.)

From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.

In studies involving the fractionation of blood phosphate compounds by means of hydrolysis with N HCl certain difficulties were encountered in the determination of the resulting orthophosphate by Kuttner's colorimetric procedure.[1] The results were usually too low, and analyses of known quantities of P under similar conditions likewise gave values below those expected. Although inclined to attribute our difficulties to the losses of HCl during hydrolysis, we had the same trouble even when we prevented such alterations in concentration. We had no more trouble when we substituted N H_2SO_4 for the N HCl. Our analyses on known quantities of P were also entirely satisfactory when H_2SO_4 instead of HCl was used for the hydrolysis. This led us to investigate the probable influence of different salts in high concentration on the orthophosphate values determined by the Kuttner method. We discovered subsequently that Rimington[2] had already pointed out the effect which the concentration of various salts used as anticoagulants may exert upon the quantitative determination of P in blood by Brigg's procedure. We, nevertheless, present these results to call attention once more to this important matter, and thus save other investigators the time and trouble it has cost us to find out this simple

[1] Kuttner, Th., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

[2] Rimington, C., *Biochem. J.*, 1924, **18**, 1297.

fact. The warning is perhaps especially needed now, inasmuch as hydrolysis by N HCl is generally employed in the study of various phosphate fractions in blood or muscle.

On neutralizing this extra acid before carrying out the colorimetric test a rather high salt concentration is produced, which in our experiments, on account of the final dilution used, amounted to 0.2 M. Not all salts interfere with the colorimetric reaction, as will be shown presently, but the chlorides and nitrates do interfere seriously. We determined in a series of special analyses the various concentrations of sulfates and chlorides at which the colorimetric determination of P is no longer accurate. These results are here summarized:

Salt	Interfering Concentrations
NaCl	0.1 M
NH_4Cl	0.2 M
$(NH_4)_2SO_4$	0.5 M
Na_2SO_4	About 3.0 M

Rimington found the NaF in a concentration of 0.01 M interfered with the phosphate determination. We also find that NaCl interferes seriously, 1.0 M concentration causing a loss of 50% in the P recovered colorimetrically. A 0.1 M $NaNO_3$ gives results that are 20% too low. The fact that the sulfate salts, especially the Na_2SO_4, interfered very much less than the chlorides, accounts for our success in using N H_2SO_4 for hydrolysis. The maximum Na_2SO_4 concentration upon neutralization of the acid and upon dilution of the solution is only 0.1 M, whereas our analyses show that this salt does not interfere even in very large concentrations and at 3 M it actually causes an increase in color by about 7%.

6100

Plasma Protein and Blood Volume.

HSIAO-CHIEN CHANG.

From the Department of Medicine, Peiping Union Medical College.

Plasma protein deficiency results in a decrease of the osmotic pressure of the blood and has been held to be part of the explanation of edema in nephrosis and certain cases of undernutrition. If this postulation be correct the capillary pressure would be in excess of the osmotic pressure of the blood and as a result of the increased

transudation a state of oligemia should be expected to prevail under such conditions. That this is actually the case in nephrosis has been shown by Darrow[1] and Waterfield.[2] The behavior of blood volume in nutritional edema has, however, not received the attention it deserves. The present report deals with the blood volume changes in both of these diseases, special attention being directed towards correlating such volume changes with the fluctuation of the plasma protein.

The plasma protein was determined by the micro-kjeldahl method and the blood volume by inhalation of carbon monoxide according to the technic reported previously.[3] Hematocrit readings were made by the method of Osgood.[4] In the cases of nephrosis the 24-hour urine output for the day of examination was also ascertained. In all, 5 cases of nephrotic kidney condition and 7 cases of nutritional edema were studied. The findings are given in Tables I and II.

On examining the data two points deserve special emphasis. First, in both diseases, when not complicated by severe anemia, the

TABLE I.
Blood Volume and Plasma Protein Findings in 5 Cases of Nephrosis.

Case No.	Date	Wt. kilo	B. M. R. %	Urine output in 24 hr. cc.	Plasma protein %	Blood Vol. Total cc.	Blood Vol. Per sq.m. cc.	Red cell volume %	Remarks
1	March 9, 1928	91.4	— 5.0	580	4.70	3690	1775		
	March 30, 1928	90.3	+ 1.4	1380	5.20	4215	1970		
	May 1, 1928	52.8	+ 0.5	1950	6.90	5025	3030		
	October 4, 1929		+ 8.8		6.60	5500	2895		Recovered
	October 17, 1930	67.6	+ 6.7	900	6.44	5430	2920	39.5	
2	January 16, 1928	61.8	—20.0	230	4.40	2918	1757		
	March 3, 1928	53.7	+13.4	1220	5.60	3835	2300		Improved
	May 2, 1928	60.9	—26.4	1140	5.50	3700	2200		
3	January 16, 1930	65.5	—41.0	1415	3.46	3155	1793	38.8	
	February 19, 1930	55.4	—27.8	1350	4.00	3540	2172	32.3	
	April 8, 1930	49.2	—16.7	1130	4.38	3670	2370	32.5	
4	October 17, 1929	45.2	—15.4	1630	4.68	4255	2820	39.6	
	December 5, 1929	43.0	— 2.7	765	3.99	3202	2180	35.0	Became
	December 23, 1929	44.8	—16.2	1100	3.81	3580	2370	37.5	worse
5	May 5, 1931	26.7	— 9.0	2500	4.57	1564	1580	37.5	
	May 19, 1931	27.9	— 9.8	790	4.88	1683	1700	37.0	
	June 3, 1931	26.0	—16.6	560	4.89	1638	1706	39.5	
	January 23, 1932	30.8	— 0.3	1450	5.83	1962	1886	37.5	

[1] Darrow, D. C., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 740.

[2] Waterfield, R. L., *J. Clin. Invest.*, 1931, **9**, 589.

[3] Chang, H. C., and Harrop, G. A., Jr., *J. Clin. Invest.*, 1928, **5**, 393.

[4] Osgood, E. E., *Arch. Int. Med.*, 1926, **37**, 685.

TABLE II.
Blood Volume and Plasma Protein Findings in 7 Cases of Nutritional Edema.

Case No.	Date	Wt. kilo	B. M. R. %	Plasma protein %	Blood Vol. Total cc.	Blood Vol. Per sq.m. cc.	Red cell volume %	Remarks
1	September 25, 1929	41.7	— 5.9	5.09	2560	1821	33.5	
	November 22, 1929	38.8	+13.4	6.56	3715	2925	32.5	
	December 6, 1929	41.4	+13.5	5.90	3550	2670	30.8	
2	April 2, 1930	49.0	—10.8	3.77	2975	1893	41.0	
	May 20, 1930	46.2	+13.7	6.87	3570	2410	40.4	
3	March 31, 1931	46.7	—11.5	5.77	3590	2163	36.8	
	June 9, 1931	50.5	+ 3.7	6.12	4240	2632	38.8	
4	February 17, 1930	41.4		4.07	3335	2315	30.6	Secondary
	March 3, 1930	43.1	— 1.3	5.81	3835	2610	30.8	anemia
5	January 21, 1930	28.4	— 5.5	5.34	2650	2430	26.2	Secondary
	February 21, 1930	30.8	+22.6	5.92	2910	2620	29.4	anemia
	March 7, 1930	32.0			2906	2570	37.8	
6	November 5, 1930	25.2	—11.7	5.37	1950	1930	19.5	Anemia
	May 6, 1931	29.1	— 3.9	6.20	2085	1905	35.2	
	June 26, 1931	34.0	+12.6	6.44	2600	2260	32.8	
	July 29, 1931	37.9	+19.9	6.57	2720	2266	34.8	
7	September 24, 1930	27.1	—22.8	3.46	1510	1510	35.4	
	March 24, 1931	24.9	—15.6	5.89	1612	1662	35.3	
	April 10, 1931	27.4	+ 0.1	6.08	1850	1832	31.4	
	May 1, 1931	28.5	—17.6	6.38	2048	1968	33.5	
	July 2, 1931	31.4	+ 5.0	6.79	2175	2015	37.7	
	July 17, 1931	32.0	+ 0.3	6.86	2355	2160	36.7	

blood volume expressed in cc. per square meter of the surface area (calculated from patient's height and weight) was very much lower than the normal standard determined by the same method.[3] This is in agreement with the previous work on nephrosis[1, 2] and further corroborates the belief that the edema of these 2 diseases may be placed under the same category. Secondly, any change in the plasma protein concentration was reflected in nearly every case by a corresponding variation of the circulatory volume and when an increase of the plasma protein took place, usually resulting in an improvement or disappearance of the edema, then the blood volume almost invariably returned towards the normal range. As the cell volume suffered only very slight changes the volume variation seemed to be chiefly confined to the plasma.

No definite correlation between the volume fluctuation and the extent of diuresis was evident in any of these patients with nephrotic kidney disease.

In conclusion one may assume that the plasma protein concen-

tration among many others is one of the regulating factors of the circulatory volume. Deviation from normal on the part of the former may be attended by a disturbance of the latter. This is well exemplified in such pathological conditions as nephrosis and nutritional edema.

6101

Experimental Production and Cure of Jejunal Ulcers.

JAMES C. OWINGS AND IVAN H. SMITH. (Introduced by F. C. Lee.)

From the Surgical Hunterian Laboratory, Johns Hopkins Medical School.

Twenty-six animals were operated upon for the production of jejunal ulcers using a slight modification of the technic of Mann and Williamson[1] for depriving the jejunum of its normal alkalinity.

In each case the duodenum was transected between the pylorus and the ampulla of Vater and both ends inverted with silk. The jejunum was then divided a few inches beyond Treitz's ligament and again both ends inverted. Following this a gastro-enterostomy was performed between the distal end of the jejunum and the stomach and an enteroenterostomy performed between the closed duodenojejunal loop and the upper ileum about 24 inches below the gastroenterostomy, allowing the bile, pancreatic juice and duodenal secretions to be emptied back into the small bowel at this point. These changes were made in the original procedure because it was felt that the nutrition of the animals would be better preserved if the drainage were done higher in the small intestine, thereby eliminating this factor from the ultimate results. Also since a lateral anastomosis has a better blood supply than one made end-to-end, there would be less chance of criticism on this score as a possible source of ulcer formation.

Ten of these animals formed typical chronic ulcers varying in size from ½ to 2½ cm. in diameter. The period necessary for the formation of the ulcers varied from 42 to as long as 428 days with the average of 118 days. The ulcers were multiple in 3 cases. They always formed on the wall of the jejunum opposite the gastroenterostomy stoma and never involved the suture material which was of silk throughout, except in one instance, where the edge of a very large perforating ulcer touched it in one spot. The animals were

[1] Mann, F. C., and Williamson, C. S., *Ann. Surg.*, 1923, **77**, 409.

explored every 2 weeks up to 6 weeks and then once a month until either an ulcer was found or they died from some other cause. Sixteen of the 26 died from extraneous causes at an average of 26 days postoperative without ulcer formation.

Three of the 10 dogs that formed ulcers died before any attempt of cure could be made; one died from hemorrhage, and 2 from hemorrhage with partial biliary obstruction. In the other 7 the bile, pancreatic juice, and duodenal secretions were poured back over the area of ulcer formation as soon as it was discovered. In order to do this the lateral anastomosis was taken down and an end-to-side union made between the duodeno-jejunal loop and the stomach. In 4 cases the ulcer completely healed, in 42, 56, 61, and 83 days respectively. In 2 it was only partially healed when the dogs died, one from distemper at 19 days, and the other from partial biliary obstruction with jaundice at 153 days. The 7th dog showed no disposition to heal and died from hemorrhage on the 13th day following the second operation. In 2 of the dogs where the ulcer had healed completely, the digestive juices were again short-circuited. In the 1st dog the ulcer reformed, perforated, and caused the death of the dog from peritonitis on the 12th postoperative day. The second attempt was a failure due to a faulty suture line, causing the death of the dog on the 4th day after operation. The general condition of the dogs remained excellent until an ulcer appeared. From this time on they rapidly lost weight and became anemic, but gained weight during the healing period after the second operation.

6102

The Etiology of Duodenal Ulcers.

JAMES C. OWINGS AND IVAN H. SMITH. (Introduced by F. C. Lee.)

From the Surgical Hunterian Laboratory, Johns Hopkins Medical School.

Since the original work of Mann and Williamson[1] much has been written concerning the experimental production of duodenal ulcers by depriving the duodenum of its normal alkalinity. It, therefore, becomes of interest to know if any one of the 3 secretions, bile, pancreatic juice, or intestinal juices, is specifically responsible for protection against ulcer formation, or whether the presence of all of them is necessary.

[1] Mann, F. C., and Williamson, C. S., *Ann. Surg.*, 1923, **77**, 409.

In an attempt to determine this point 3 series of experiments were done. In the first series 5 operations were performed under aseptic technic and the common bile duct isolated, tied, and divided in each case. Following this a cholecyst-enterostomy was performed at a point on the small bowel about 18 inches below Treitz's ligament, thereby excluding the bile from the duodenum and draining it into the jejunum at this point. The anastomoses and closures were done with silk. Two of these 5 animals developed an acute duodenal ulcer at 76 and 119 days postoperative respectively. In each case the ulcer was situated exactly at the pyloric ring and was about 3 mm. in diameter. Both died of hemorrhage into the bowel without warning and the ulcer was only discovered at autopsy. Pathologically these ulcers were very different from chronic ulcers produced by duodenal drainage. They were smaller, more punched out, showed no sign of secondary infection, had hemorrhagic bases ulcerating into a vessel in each case, and presented no evidence on the serosal surface by which ulcer formation could have been predicted. Microscopically, they showed very little infiltration and no scar tissue formation. Both animals were in good general condition at the time of death. The 3 others were well nourished and showed no sign of ulcer formation at the date when they were sacrificed, 234, 236, and 239 days postoperative respectively.

Microscopic sections taken of the liver and gall-bladder in this series showed slight low grade infection of the gall-bladder but no cholangeitis. It was felt that this presented an interesting point, in view of the high percentage of infection reported clinically following cholecyst-enterostomy. It is possible that our good results were due to the small openings used in our anastomoses, for the average measurement of these openings at autopsy was only 2.3 mm.

In a second series of experiments an effort to determine the effect of loss of pancreatic juice was made. The accessory pancreatic duct was located, ligated, and divided, and the main duct was isolated and transplanted into the jejunum 18 inches below Treitz's ligament. The implantation of the duct and all closures, both of bowel and abdominal wall, were done with silk. These dogs were then allowed to go for an average of 123 days, being fed the usual laboratory diet. They were explored every 3 weeks during this period and at no time was there any evidence of ulcer formation. Their nutrition and condition in general remained excellent.

The third series of experiments was performed on these same animals. It consisted of ligation and division of the common bile duct with concomitant cholecyst-enterostomy about 2 inches below the

point where the pancreatic duct had already been transplanted. This deprived the duodenum of both bile and pancreatic juice, leaving it only duodenal juices for protection against the acid gastric secretion. The dogs were now allowed to go on for an average of 78 days, during which period they were explored several times with no evidence of ulcer formation. At the end of this time when they were sacrificed their nutrition was good, there was no anemia, and no damage to the mucosa of the duodenum.

The number of these experiments is too small and the period of time too short to draw any definite conclusions, especially in view of the inconstant results, but they suggest that the bile is the most important of the 3 factors.

6103

A New Method for Determining the Fragility of Red Blood Cells.

BRUCE K. WISEMAN AND OLGA S. BIERBAUM. (Introduced by C. A. Doan.)

From the Department of Medical and Surgical Research, The Ohio State University.

Prevailing methods for the determination of the fragility of erythrocytes are based upon the theory of their hemolytic stability when brought into contact with chemicals such as saponin and bile salts, with specific sera, or with hypotonic salt solutions. Theoretical as well as practical considerations have directed the development of these studies toward improving the technique for determining the resistance of the red blood cells to solutions containing different concentrations of various salts. Of the many variations in the original method of Ribierre,[1] that of Simmel,[2] or one of its modifications,[3] appears to be the best. However, the necessity of freshly prepared hypotonic salt solution of very exact concentrations, together with the labor in using the counting chamber and the necessity for setting up a control test with a known normal blood at each observation, are definite disadvantages.

We have devised a fragility test in which the difficulties mentioned are largely eliminated. The testing of the relative fragility of the

[1] Ribierre, P., *L'hemolyse et la mesure de la résistance globulaire; application à l'étude de la résistance globulaire dans l'ictère. Thèse de Paris*, 1903, No. 154.

[2] Simmel, H., *Arch. f. klin. Med.*, 1923, **142**, 252.

[3] Waugh, R. T., and Chase, W. J., *J. Lab. and Clin. Med.*, 1928, **13**, 873.

patient's cells in varying dilutions of his own plasma is the essential principle introduced in this new method.

Technique. Test tubes of 20-30 cc. capacity and capable of withstanding the high speed of the centrifuge are fitted with rubber stoppers. To each is added 2 mg. of powdered heparin, an amount sufficient to prevent the clotting of 10 cc. of blood for approximately 24 hours. After weighing out this quantity a few times to visualize the approximate volume involved, the quantity of heparin added to each test tube may be estimated without disturbing the accuracy of the test. Ten cc. of blood are drawn from one of the arm veins, using a dry (not rinsed in salt solution) 10 cc. syringe and needle. After discharging the blood into the heparin-containing tube, the latter is rocked back and forth for about one minute and put aside until the test is to be set up.

Thirteen agglutination tubes (11 mm. x 75 mm.) are placed in an appropriate rack. Tube No. 13 is half-filled with the whole blood. The remainder of the sample is centrifuged, and the clear plasma removed with a Wright's capillary pipette. Two-tenths cc. of the plasma is transferred into each of the tubes Nos. 1-12, inclusive, using a 1 cc. serum pipette graduated in 100 divisions and equipped with a rather sharp delivery end. Various quantities of fresh glass-distilled water are then added to each of these 12 tubes in amounts as shown in Table I, and mixed with the plasma previously added. Finally, 0.02 cc. of the whole blood from tube No. 13 is pipetted into each tube, and the tube immediately shaken. After standing 2 hours or more at room temperature the tubes are examined and the points at which hemolysis begins and is complete

TABLE I.
Plasma Dilutions with Calculated Equivalents in Salt Concentrations.

Tube No.	Dist. Water cc.	Salt Equivalents†	Tube No.	Dist. Water cc.	Salt Equivalents†
*	.06	.707	5	.28	.396
*	.08	.660	6	.30	.380
*	.10	.618	7	.32	.366
*	.12	.582	8	.36	.341
*	.14	.550	9	.40	.319
*	.16	.521	10	.44	.300
*	.18	.495	11	.48	.282
1	.20	.471	12	.52	.267
2	.22	.450	*	.56	.253
3	.24	.430	*	.64	.230
4	.26	.412	*	.72	.210

All tubes have 0.20 cc. plasma and 0.02 cc. whole blood added to the above amounts of distilled water to complete the test.

* Tubes not numbered are set up only when needed for exceptional cases.

† See footnote p. 837.

are recorded. At this reading it is also noted whether the plasma in tube No. 13 is free from spontaneous or traumatic autohemolysis —if not, the results must be rejected, new blood obtained, and the test repeated. It is best to use the same pipette for measuring the plasma, distilled water and blood.

Application of the Test. Blood samples have been secured from 50 healthy individuals varying from 10 to 60 years. In no case has hemolysis been noted in tubes 1-3 inclusive, nor was a higher dilution required for complete hemolysis than that represented by tube No. 10. It is, therefore, believed that when hemolysis occurs in more concentrated plasma than that represented by tube No. 4, pathological fragility is indicated, and when hemolysis has not started in the dilution represented by tube No. 5, or is not complete in that of tube No. 10 an abnormal increase in erythrocyte resistance is present. The salt equivalents* for the limits of normal as established by this test are 0.300-0.412.

Inter-plasma controls have been made on normal and pathologic bloods to determine definitely whether the increased ease of hemolysis in certain individuals is a property of cells or plasma. While the important determination is of course the effectiveness with which the homologous red blood cells resist the hemolyzing environment of their own plasma, which this test assures quite irrespective of the salt-equivalent-resistance-value, it has been of interest to analyze the constancy of the isotonicity of the blood plasma from individual to individual. In no single instance in the 100 bloods studied to date, including both normal and pathologic fragility ranges, has the blood plasma influenced the index of hemolysis for homologous or isologous erythrocytes.

Among the 50 individuals, representing a variety of diseases, from whom blood samples have been tested, there were 3 cases of congenital hemolytic icterus. As originally tested in dilutions of their own plasma these erythrocytes all showed a marked increase in fragility above the established normal values. The cells from the cases of congenital hemolytic icterus, when tested against plasma from normal individuals, showed hemolysis within exact limits established by tests with their own plasma. Conversely, the cells from the normal individuals were tested in the plasma from the cases with hemolytic jaundice, but no increase of fragility was demonstrated.

* These values are calculated as percentage of sodium chloride, assuming that 0.9 gm. sodium chloride per 100 cc. distilled water yields a solution isotonic with mammalian blood.[4]

[4] Starling, E. H., Principles of Human Physiology, Philadelphia, Lea and Febiger, 5th Edition, 1930.

These results showed that the cells both from the normal and jaundiced patients retained their respective characteristic fragilities without reference to the source of the plasma.

Using the technique described in this paper, a detailed study of the index of hemolysis in both normal and diseased individuals will be presented elsewhere.

6104

An Intravascular Lesion in Poliomyelitis Induced by Feeding in Macacus Cynomolgus.

R. S. SADDINGTON. (Introduced by Simon Flexner.)

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Wickman[1, 2] dwelt at some length upon the lesions of the central nervous system associated with acute poliomyelitis, and as a result of the accuracy of his original observations his descriptions of the changes encountered have been little altered in subsequent years. Wickman noted that the vascular lesions of acute poliomyelitis were more marked in the veins than in the arteries, and that the frequently encountered round cell infiltration was situated in the lymph channels of the vessel wall. It was also recognized, however, that the lymphocytic infiltration was often of sufficient intensity to extend beyond the adventitial limits of the vessels into the surrounding tissues. Flexner and Amoss[3] pointed out that in cases of acute poliomyelitis induced by intravenous administration of virus the vascular lesions were more extensive than in instances of infection by other routes.

The lesion described below was encountered in the meningeal vessels of a *Macacus cynomolgus* which developed acute poliomyelitis after having been fed with virus-infected milk. The material used was a 10% milk suspension of recently glycerinated brain and spinal cord from monkeys which had succumbed to typical poliomyelitis. Six daily feedings of 30 cc. were administered by mouth with the aid of a medicine dropper, the milk being fed slowly so that the monkey could swallow it easily.

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[1] Wickman, I., *Studien über Poliomyelitis acuta.* Karger, Berlin, 1905.

[2] Wickman, I., *Die akute Poliomyelitis bzw. Heine-Medinsche Krankheit.* Springer, Berlin, 1911.

[3] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, **20**, 249.

Symptoms were first apparent 11 days after the last feeding, at which time the animal moved about slowly and showed obvious weakness of the left leg. During the course of the illness the left leg became completely paralyzed and the right leg became quite weak. On post mortem examination a moderate congestion of the central nervous system was observed. Microscopically, lesions typical of poliomyelitis were found in the pons, in the medulla, and in the lumbar segments of the spinal cord. Changes in the dorsal and cervical segments were minimal.

Of particular interest was the peculiar inflammatory reaction involving the meningeal veins at the various levels of the central nervous system exhibiting parenchymal lesions. The vein walls were infiltrated by small and large lymphocytes. These were not uniformly distributed about the circumference of the affected vein, as is usually the case, but were collected in a nodular or elliptical mass along only one portion of the vessel wall. The infiltrating cells lay not only in the vascular lymphatics, but extended into the adventitia and also invaded the inner portions of the vein wall, lymphocytes lying in some instances just beneath the endothelium of the vein involved.

This peculiarly focal reaction was most sharply defined in the walls of the anterior and posterior spinal veins at the lumbar level of the spinal cord. Here the intensity was such that an actual convexity was produced upon the inner surface of the vein. In such an area large numbers of lymphocytes were so massed together with the pale swollen endothelial cells of the lymphatic channels that structural detail was concealed. The endothelial cells of the intima of the affected veins were swollen in contrast to those of the arterial intima. The arteries showed surprisingly little change.

The interest of the lesion lies in its strict limitation to the veins of the meninges, in its sharply defined elliptical arrangement, in its peculiar focal intensity and in the fact that it was intravascular in contrast to the more frequently encountered perivascular response.

6105

A Method of Obtaining Large Amounts of Rickettsia Provaceki by X-ray Radiation of Rats.

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Eventual interepidemic control of typhus fever will depend upon the effective sanitary utilization of the epidemiological facts now available. Even with our almost complete knowledge of transmission and animal reservoir, however, the circumstances under which typhus epidemics occur usually exclude any hope of adequate control. Students of typhus, therefore, have long felt that in this disease particularly a method of specific prophylaxis was needed.

The *Rickettsia provaccki* may now be accepted as the established etiological agent, and all rational methods of vaccination must be based on (1) active immunization with Rickettsia, killed, attenuated or in subinfectious doses; (2) immunization with living Rickettsia, sensitized or neutralized with convalescent or immune serum; and (3) passive prophylaxis with such serum alone.

The ideal method at which first attempts should be aimed is active immunization with killed Rickettsia material. The first fact to be ascertained is whether or not such a thing is possible.

Active immunization of guinea pigs with carbolized Rickettsia was first accomplished by da Rocha-Lima,[1] confirmed by Weigl,[2] Breinl,[3] and Rosenberger.[4] Doerr and Schnabel[5] obtained negative results, possibly, as Otto[6] suggests, because of deterioration of their vaccines. This is rendered likely by Kemp,[7] who found that our own early vaccine material lost potency within about 4 weeks. The above writers, as well as Otto, believe that the results obtained with louse vaccines, in contrast to the negative attempts made with virulent tissues—brain, blood, etc.,—are attributable to the high concentration of Rickettsia in the louse vaccines.

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[1] Da Rocha-Lima, *Mediz. Klinik.*, 1917, 1147; also *Münch. Med. Woch.*, 1918 **2**, 1454.

[2] Weigl, *Mediz. Klinik.*, 1924, 1046.

[3] Breinl, F., *Z. f. Immunitätsforsch.*, 1924, **41**, 97.

[4] Doerr u Schnabel, *Wien. Klin. Woch.*, 1919, 523, 891.

[5] Otto, R., and Munter, H., *Fleckfieber, in Kolle u Wassermann Hankbuch*, 3rd Edition, 1930, **8**, 1200.

[6] Rosenberger, cited from Otto and Munter, *loc. cit.*

[7] Kemp, H. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 353.

In our first vaccinations with formalinized tunica material of Mexican typhus with Batchelder[8] we were encouraged chiefly by the results of Spencer and Parker[9] and of Conner[10] with Rocky Mountain Spotted Fever, which favored the belief that active immunization with dead Rickettsia was feasible provided a sufficient concentration could be attained.

We proceeded, therefore, with the production of vaccine materials from tunica tissues of guinea pigs infected with the Mexican strain, with encouraging results. We used 0.2% formalin as the sterilizing agent, because formalin seemed, in our studies with bacteria, to exert the least denaturizing effect upon antigen, and because Dunkin and Laidlaw[11] had found it suitable in experiments with a filterable virus. Subsequently, we carried out concentration of Rickettsia bodies in the peritoneal cavities of infected rats in which resistance had been reduced by scorbutic diets and by injections of benzol in olive oil.[12] We showed that increased resistance and not infrequently complete immunity can be obtained in guinea pigs by vaccination with large doses of such formalinized suspensions.

Kemp[6] has confirmed the basic fact that formalinized tunica virus can actively immunize guinea pigs, and has pointed out the relative instability of the vaccine.

We ventured to make no predictions in our earlier papers of the possible applicability of this method to the prophylaxis of man. Casco[13] has tested on man our later vaccine, the formalinized exudate of benzolized and infected rats. His results are not discouraging, perhaps encouraging, since only 3 out of 11 vaccinated individuals, and 2 out of 3 controls developed typhus on inoculation with virus. But the numbers are too small and the histories of native Mexicans too uncertain to permit ourselves too much happiness on these figures. What is more encouraging than the actual reinoculation results is the development of Weil-Felix reactions in a large proportion of these and of other vaccinated people observed by Casco.

The present aspects of the problem may be summarized as follows: 1. Active immunization with killed typhus virus is possible.

[8] Zinsser, H., and Batchelder, A. P., *J. Exp. Med.*, 1930, **51**, 847.

[9] Spencer and Parker, *U. S. Public Health Rep.*, 1926, **41**, 35.

[10] Conner, *J. Immunol.*, 1924, **9**, 4.

[11] Dunkin, G. W., and Laidlaw, P. P., *J. Comp. Path.*, 1926, **39**, 201, 213.

[12] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649.

[13] Sanchez-Casco, R., Thesis, 1932, Faculdad de Medicina, National University of Mexico.

2. The immunizing properties of a vaccine seem to depend directly upon the concentration of Rickettsia contained in it.

From this point of view we have attempted to evolve a reliable method for obtaining still higher concentrations of Rickettsia from the Mexican strain. The benzol method was the one employed for Casco at the Mexican Hygienic Institute. While this procedure, especially when combined with the subjection of the inoculated rats to a temperature of about 10°C., usually gave excellent results, we found on consistent study throughout a year that it was subject to variations and that it occasionally failed entirely during the hot months. There is undoubtedly a seasonal fluctuation of typhus susceptibility both in rats and in guinea pigs which complicates experimental procedures.

For these reasons, we attempted many modifications of the procedures for Rickettsia concentrations. We finally succeeded in developing a method which has been carried out on more than 50 rats, and has yielded, with a regularity of approximately 90%, a Rickettsia suspension always equal to the best materials obtained by the benzol procedure, and occasionally superior, even simulating the concentration of bacteria in cultures.

The method depends upon the use of X-rays. Actual radiation was carried out for us at the Huntington Memorial Hospital by Dr. Richard Dresser, without whose cooperation the work would have been impossible. We abstain from reporting preliminary studies and comparative blood counts. As far as the changes in circulating white cells are concerned, our results are consistent with those of others.

The ideal aimed at for our purposes was radiation with short wave lengths sufficiently severe to affect the resistance of the animal, but permitting it to live long enough for adequate multiplication of the Rickettsia. We used rats, guinea pigs and a few rabbits. The exposure which has given us the best results with rats is, under Dr. Dresser's control, as follows: 170 K. V. constant potential; 80 cm. distance; 0.5 mm. copper filter plus 4 mm. celluloid; 8 milliamperes; the effective wave length 0.160 Angstrom units; intensity 10 "r" units per minute. The most suitable time for the exposure of rats has been found to be one hour—*i. e.*, 600 "r" units.

The rats are intraperitoneally inoculated immediately after radiation with a suspension of a Mexican typhus tunica. The material for inoculation need not be richer in Rickettsia than the average obtained in routine transfer. Control rats treated by X-ray in the same way begin to get sick on the 4th or 5th day, but may live 6, 7,

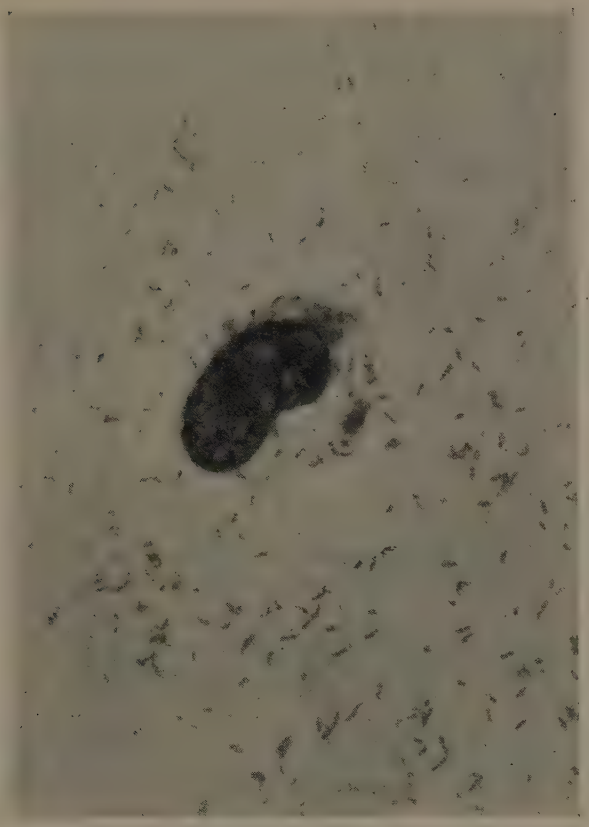

FIG. 1.
Rickettsia in Peritoneal Exudate of X-rayed Rat. Average yield.

or 8 days. The inoculated rats are sicker than the controls by the 3rd day, when some of them may die. This is too early for an adequate yield of Rickettsia. On the 4th day many of the rats appear ill, and by the 5th day they have begun to die. According to the condition of the rats, they are killed on the 4th or 5th day. The skin is dissected from the abdomen and the peritoneum washed and gently scraped with 0.2% formalin salt solution, as in the case of the benzol rats.

The large number of extracellular Rickettsia are not, in our opinion, organisms that have developed outside of the cell. If one examines rats treated in the above manner a day earlier than the optimum, one finds few extracellular organisms, but enormous quan-

tities enclosed within cells. The bursting of these cells results in the large numbers of extracellular organisms.

This method of vaccine production should be undertaken only by immunes, since it is next to impossible to avoid eventual infection.

We do not believe that it will be possible to obtain the results described unless the Mexican strain is used. But since the Mexican vaccine gives a definite though imperfect immunity to the European virus, a sufficient cross immunization can be expected to justify the testing of this method against the European disease.

We have not been able to obtain results in guinea pigs analogous to those obtained in rats. Experiments with rabbits are being made, but the proper exposure to X-ray has not yet been ascertained. Rabbits may die within 24 hours of an exposure to X-ray which rats survive for a week.

6106

Influence of the Concentration of Iodoacetic Acid Upon Excitability and Chemical Changes in Muscle.

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The report of Lundsgaard that muscle from animals treated with iodoacetic acid gives large numbers of relatively normal contractions without perceptible lactate accumulation stimulated us to study the phenomenon in order to rule out several possible sources of error. It seemed possible that iodoacetic acid might accelerate the removal of lactate rather than prevent its formation, and that the poison served to diminish the irritability and the number of contractions rather than influence the lactic acid formation. In studying this problem an important relation between concentration of iodoacetic acid and the rate of lactic acid formation has appeared.

Iodoacetic acid was dissolved in Ringer's fluid, and injected, unneutralized, into the ventral lymph sac, in doses ranging from 1 part in 5,000, on the basis of body weight of the frog, to 1 part in 30,000. Similar concentrations were used in experiments in which iodoacetic acid was added to normal intact muscle or to muscle brei.

Numerous experiments upon the effect of changing the concentration of iodoacetic acid were made. At 1:5,000 lactic acid production is completely inhibited. At 1 to 30,000 there is a consider-

able production. The muscles poisoned at less concentration went into rigor early and were obviously not normal although there was lactic acid production. In order to analyze the effect of differing poison dosage, the fate of lactic acid in the post-contraction period was studied. After stimulation to fatigue by single shocks spaced one second apart normal muscle shows no "recovery" lactate production. The fact is well known. But we found that iodoacetic-poisoned muscle shows a very large "recovery" lactate accumulation at the low dosage, and none at high.

In doses larger than 1/30,000, no lactate accumulation occurred, regardless of the interval allowed. These observations show that small doses of iodoacetic acid, which do not inhibit completely the accumulation of lactate, decrease the velocity of its formation. Extending this line of reasoning, the effect of high dosage is to slow the process to the extent of a practical stoppage.

In view of the fact that iodoacetic acid eventually renders muscles non-irritable it was conceivable that the lack of lactic acid production in them might be related to their inability to respond to excitation. A crucial test of this possibility lies in the study of a condition such as chloroform rigor, which does not depend on natural irritability. Iodoacetic acid poisoned muscles are found to go into strong chloroform rigor without demonstrable production of lactate. It seems inescapable that non-irritability is not responsible for the failure of lactate production.

We were also interested in knowing whether or not iodoacetic acid might accelerate the removal of lactic acid rather than inhibit its formation.

TABLE I.
Influence of Concentration of Iodoacetic Acid upon Lactate Production.

Experiment		Iodoacetic Injected	Min. after Injection	Procedure	Lactic Acid Found %
27	A	1/5,000	37	resting	.019
	B			fatigued	.013
29	A	1/15,000	35	resting	.015
	B			fatigued	.019
34	A	1/20,000	30	resting	.028
	B			fatigued	.033
31	A	1/30,000	30	resting	.091
	B			fatigued	.195

TABLE II.
Influence of Iodoacetic Acid upon Rate of Lactate Production of Fatigued Muscle.

Experiment		Iodoacetic Injected	Min. after Injection	Lactic Acid Found %	
15	A	1/30,000	35	.054	Frozen immediately.
	B			.128	In N_2 20 min. after stim.
17	A	1/6,000	40	.018	Frozen immediately.
	B			.018	In N_2 20 min.
20	A	Control	—	.242	Frozen immediately.
	B			.256	In N_2 20 min.
21	A	Control	—	.210	Frozen immediately.
	B			.215	In N_2 20 min.

Muscle brei of thigh muscle taken from injected frogs uniformly showed complete inhibition of lactic acid formation after doses of 1/5,000, 1/6,000, or 1/15,000, but incomplete inhibition after doses of 1/30,000. The rate of lactic acid production in brei of muscle is slowed in proportion to the concentration of iodoacetic acid.

Summary. 1. There is conclusive evidence that iodoacetic acid slows the rate of lactic acid production in the anaerobic recovery period, and the slowing is in proportion to the concentration of the poison. 2. Iodoacetic acid does not accelerate removal of lactate under anaerobic conditions.

6107

Sexual Reactions of Certain Anurans after Anterior Lobe Implants.

HALCYON W. BARDEEN. (Introduced by M. F. Guyer.)

From the Department of Zoology, University of Wisconsin.

It is well known that homoplastic implants of the anterior lobe of the pituitary induce sexual precocity in many animals. The present investigation continues the work of Wolf,[1] on frogs. The same technique of injection is used. Since, by giving daily implants of anterior lobe of the pituitary to mature frogs that had ovulated normally in April, it was possible to induce a second ovulation as early as October, it seemed of interest to determine how soon a sec-

[1] Wolf, O. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 692.

ond ovulation could be secured by this method, following a previous artificially induced one. By thus beginning the experiments with an artificially induced ovulation, the season of the year is apparently negligible, and the exact time of the first ovulation is accurately recorded.

The frogs were heavily parasitized and many died of "red-leg". Fifty mature female leopard frogs *(Rana pipiens)*, were given implants of 2 anterior lobes of the pituitary daily. Only 5 survived the entire experiment. After about the fifth implant, 44 were still living and had ovulated. The number of eggs discharged in every case exceeded four thousand. The second day after ovulation, an observational operation was performed in many to see if any mature ova remained undischarged. In no case were there more than 18 mature ova in the body cavity, ovaries, oviducts or uteri. (Postmortem examinations made on those that died of red-leg after ovulation confirmed these findings.) After 2 months, only 5 frogs were still living. Daily implants of anterior lobe were again given. After the third implant, one of the frogs died of red-leg. Postmortem investigation revealed the eggs were mature and the oviducts were swollen and ready for ovulation. After the eighth implant, the remaining 4 experimental frogs had again ovulated. The number of ova discharged were in each case more than four thousand. Some of these eggs were fertilized by males stimulated with anterior lobe implants, and went entirely through metamorphosis.

Can the second artificially induced ovulation be speeded up still more by daily implants of anterior lobe of the pituitary? Out of 8 frogs only 2 survived. After the first ovulation, one of these was given one anterior lobe injection daily, and on the 26th day, the amount injected was increased to 2 anterior lobes a day. On the 29th day the second animal was also given 2 anterior lobe injections. This daily treatment was continued until the 39th day when both animals were killed. Postmortem inspection showed that the ova in the ovaries of each frog were only medium sized, with more development in the frog which had not received the implants daily after the first ovulation. Judging from this limited number of animals, there is no effect in speeding up the rate of development of the ova in the ovaries by such implants, although obviously more animals must be used in such an experiment before positive conclusions can be drawn.

Experiments were also carried on which duplicated the findings of Houssay, Giusti, and Lascano-Gonzalez,[2] in inducing ovulation

[2] Houssay, B. A., Giusti, L., and Lascano-Gonzalez, J. M., *Rev. Soc. Argent. Biol.*, 1929, 5, 397.

in toads *(Bufo americanus)*, in that only homoplastic implants of anterior lobe substance were effective. As many as 2 implants of frog anterior lobes were given daily for 15 days with no result. On the other hand, homoplastic implants readily induced ovulation.

Contrary to the findings of Wolf,[1] it was possible to induce male frogs to fertilize eggs at any time of the year by daily implants of anterior lobe of the pituitary. In most cases only one implant was necessary to induce trilling, croaking, clasping, and discharging of the sperm. However, 2 implants were sometimes required. It was also found that ovulation in the females was hastened by the clasping of the male.

Several other facts of interest were noted. Contrary to the findings in the case of rats by Evans and Simpson,[3] the speed of the reaction seemed to be uninfluenced by the sex of the animal from which the anterior lobe substance was taken. When the frog ovulates normally, practically all of the eggs are discharged successively at one laying. The few mature eggs that remain are discharged within a few days. The thousands of ova in the ovaries develop simultaneously, and when all are mature, they are discharged at the same time. Giving 2 anterior lobes at each injection proved more satisfactory both as to the time of the reaction and as to the number of anterior lobes necessary to induce the reaction.

6108

Avitaminosis. X. Further Studies on the Effect of Vitamin B Deficiency on Lipid Metabolism.*

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Sure and Smith[1, 2] have reported preliminary results on the influence of vitamin B deficiency on the concentration of lipids in the blood of the albino rat, indicating that lipemia is a symptom complex in this avitaminosis. Further detailed studies from this laboratory,†

[3] Evans, H. M., and Simpson, M. E., *Am. J. Phys.*, 1929, **89**, 375.

* Research paper No. 267, Journal Series, University of Arkansas.

[1] Sure, B., and Smith, M. E., *J. Am. Med. Assn.*, 1931, **97**, 301.

[2] Smith, M. E., and Sure, B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 158.

† All of the biochemical work on blood changes in avitaminosis is being carried out in the laboratory of Agricultural Chemistry.

however, do not substantiate such findings. The reason that led us to subject to a critical analysis the lipemia in polyneuritic nursing young of the albino rat,[2] interpreted as due to vitamin B deficiency, is the fact that no appreciable changes were found in the concentration of either fatty acids, cholesterol, or phospholipids in weaned rats suffering from vitamin B (B_1) deficiency. Furthermore, an analysis of the data of Sure and Smith on lipid changes in the blood of weaned rats on diets deficient in the vitamin B complex,[3] disclosed too many great variations on the same animal from week to week, particularly in the fatty acid determination. It was discovered that carbon dioxide emanating from a close gas burner (which was being used some days during the week) was influencing the fatty acid titer considerably; therefore, all subsequent fatty acid determinations were henceforth carried out in a different room kept as free from the influence of carbon dioxide as possible, a precaution discovered in our laboratory (A. E. C.) before the same disturbing factor was called attention to in a recent paper by Himwich, Friedman, and Spiers.[4] From then on we ceased obtaining great variations in the weekly determinations of blood fatty acids. The work on the vitamin B complex was, consequently, repeated on adult rats depleted until total collapse, and even during the last stages of the avitaminosis, associated with marked inanition, no significant changes were detected in the concentration of either fatty acids or cholesterol of the blood. Such results prompted us to determine whether any lipemia is encountered in starvation, and surprisingly, no appreciable changes were detected in either fatty acids or cholesterol in full grown rats during a starvation period of 12 to 18 days.

We also repeated the work on lipid metabolism in lactating mothers and nursing young of the albino rat and have taken records of the periods when the young were nursing exclusively and when they began to partake of the maternal diet. Such observations lead us to interpret that the higher concentration of fatty acids in the polyneuritic nurslings (which takes place in certain litters and not regularly) is due to the longer periods of suckling of the pathological young compared with those receiving the maternal diet restricted to the same plane of nutrition fortified with vitamin B. No changes were found in the concentration of cholesterol between the pathological and control groups. The work on the phospholipids was not repeated, since the changes first observed were of a small degree. Since the lactating albino rat produces a milk of a fat content of

[3] Sure, B., and Smith, M. E., *Arch. Int. Med.*, 1932, **49**, 397.

[4] Himwich, H. E., Friedman, H., and Spiers, M. A., *Biochem. J.*, 1931, **25**, 1839.

31.6%,[5] the character of lipemia encountered in polyneuritic nursing young is most probably of alimentary origin, but not due to vitamin B deficiency.

6109

Dissimilarities Between Antigenic Properties of Red Blood Cells of Dove Hybrid and Parental Genera.*

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Studies by Landsteiner and Van der Scheer[1] and by Landsteiner[2] have shown that 2 different species-hybrids may be distinguished from their parental species by the use of sera immunized against the respective blood cells, with subsequent agglutinin-absorptions. The experiments to be presented involve a cross of females of the domesticated Ring dove *(Streptopelia risoria)* with males of an Asiatic genus *(Spilopelia chinensis)*, commonly called Pearlneck. Antisera were prepared by injecting rabbits with erythrocytes from individual representatives of each genus and of the hybrid. The agglutinations were performed by adding to 0.1 cc. of the immune serum in its varying dilutions (by halves) one drop of a 2.5% suspension of the red blood cells. For the absorptions, twice the volume of the serum diluted according to its original titre was added to a given volume of washed, packed red blood cells. The mixture was agitated gently at intervals, allowed to stand at room temperature for 2 hours and in the ice box overnight. All absorptions were repeated until complete at a dilution of 1:30. Readings were generally made after 2 hours at room temperature.

Without giving the inter-agglutinations of the parental genera and the hybrid, to be published elsewhere, it may be said that each

[5] Donaldson, H. H., The Rat, second edition, p. 316. Memoirs of the Wistar Institute of Anatomy and Biology, Philadelphia, 1924, 6.

* Published with the approval of the Director of the Station. This investigation was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council, grant administered by Professor L. J. Cole.

[1] Landsteiner, K., and Van der Scheer, J., *J. Immunol.*, 1924, **9**, 213.

[2] Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 981.

was readily distinguishable from the others after reciprocal absorptions had been made.

Certain serological inter-relationships of the parental genera and the hybrid are presented in the table. On the basis of these reac-

TABLE I. Agglutinations.

Immune Serum	Absorbed by cells of	Tested on Cells of Pearlneck	Ring dove	Hybrid
Pearlneck	Hybrid	Tr-40	0	0
Ring dove	Hybrid	0	Tr-60	0
Hybrid	Ring dove	240	0	240
Hybrid	Pearlneck	0	100	120
Hybrid	Ring dove and Pearlneck	0	0	60

The agglutinations of the cells were complete at the above dilutions, except as noted.
Tr = trace.

tions, it is seen that the cells of the hybrid resemble those of the Pearlneck genus more closely than those of the Ring dove. Also, the hybrid cells do not possess quite all of the substances particular to each of the parental genera.

On the assumption that the biochemical composition of the erythrocytes is determined entirely by the genic complex of the individual, evidence for which is reserved for a later publication, it is here shown that the hybrid birds present an example wherein the haploid number of chromosomes—hence the genes acting singly—has practically the same effect upon a character as the diploid number, since nearly all of the antigenic substances of both genera are present in the cells of the hybrid.

From the table, it is evident that in addition to the specific parental substances in the cells of the hybrid, a different biochemical character is present. Presumably this is a result of the interaction of the 2 different sets of chromosomes of the hybrids, or more specifically, to the interaction of the genic complexes. Each of 14 hybrids was specific for this character.

Confirmatory evidence has been found in tests involving hybrids between the common pigeon *(C. livia)* and female Ring doves. Serum immunized against the cells of the hybrids, when absorbed by the cells of both parental genera, agglutinated those of the hybrids at a low dilution.

The distributions of these hybrid substances among successive backcrossed generations from matings of each generic hybrid to Ring dove individuals are being investigated.

6110

A Positive Friedman Test in a Case of Teratoma Testis with Gynecomastia.

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(Introduced by S. Goldschmidt.)

From the Departments of Physiology and Urology, University of Pennsylvania Medical School.

Zondek,[1] Heidrich, Fels and Mathias[2] and Hady[3] have reported obtaining a positive Ascheim Zondek reaction in cases of chorionepithelioma testis.

Stimulated by these reports we have had the opportunity to study a case of testicular tumor admitted to the surgical ward of the University of Pennsylvania Hospital.

The patient, H. A., a white male, age 24, was admitted with a history of a firm mass the size of a golf ball in the right testis of two months duration. There was a slightly visible and definitely palpable intumescence and induration about 4 cm. in diameter under each areola of the breasts.

The right testicle and the distal end of the spermatic cord were removed at operation and a tumor 3 cm. in diameter involving the lower pole of the testis and infiltrating the seminiferous tubules was found.

Three months later, with no clinical evidence of the presence of metastasis of the tumor, a morning specimen of urine was obtained and 24 cc. of urine were injected intravenously into an isolated non-pregnant female rabbit according to the method described by Friedman and Lapham.[4] On sacrificing the rabbit several corpora lutea and corpora hemorrhagica were found in both ovaries resembling exactly the reaction obtained on injection of urine of pregnancy.

The pathological report by Dr. Joseph MacFarland on microscopic examination of the tissue after the test was teratoma of the testis.

In view of these results it is quite evident that this reaction may be of value in establishing a diagnosis of teratoma testis and also in the determination of the presence of metastatic tumor tissue of this character.

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[1] Zondek, B., *Chirugie*, 1930, **2**, 1072.

[2] Heidrich, L., E. Fels u. E. Mathias, *Beitrage zur Klin. Chirugie*, 1930, **150**, 349.

[3] Hady, *Zentralblatt für Gynäk.*, 1931, **55**, 912.

[4] Friedman, M. H., and Lapham, M. E., *Am. J. Obstet. and Gynecol.*, 1931, **21**, 405.

The determination of the nature of this reaction and its clinical value as an aid in diagnosis must await further study.

6111

Extirpation Experiments Upon the Pouch Young of Didelphis Virginiana.

CHARLES S. APGAR. (Introduced by Helen Dean King.)

From the Wistar Institute of Anatomy and Biology, Philadelphia.

Extirpation experiments upon the embryonic forelimb of the rat demonstrating their inability to regenerate have been described.[1] In order to carry on this type of investigation with a more primitive animal the pouch young of the Virginia opossum, *Didelphis virginiana,* were subjected to a series of experiments to determine their capacity for regeneration.

The anesthetized female with new born young was stretched dorsicumbent on an operating table and the extremities fastened down. The pouch was held open by retraction and the pouch young[2] were operated upon with fine iridectomy scissors.

These preliminary operations were made in order to ascertain whether any particular locality was preferred for such treatment. Gross examination of these specimens after fixation in Bouin's fluid showed no signs of regeneration. In these experiments 1 to 5 digits or 1 foot from a posterior limb, or 1 posterior limb, or the tail were removed from a series of 16 animals. The specimens were killed and preserved at intervals from 33 to 315 days after the operation.

Unfortunately, due to the difficulties of maintaining the opossum in the laboratory, half of the experimental material was lost. Further work is planned using the young immediately at birth and, as before, confining the experiments to the posterior region of the body, which is less developed at the time of birth.

[1] Nicholas, J. S., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 436.

[2] Hartman, C. G., *J. Morph. and Physiol.*, 1928, **46**, 1. Hill, J. P., *Proc. Zool. Soc. London*, 1917, **24**, 337. Langworthy, O. R., *J. Comp. Neur.*, 1928, **46**, 201.

6112

Effects of Ephedrine Upon Blood Lactic Acid and Respiratory Metabolism in Man

G. S. COLTRIN. (Introduced by R. G. Sinclair.)

From the Department of Biochemistry and Pharmacology, University of Rochester School of Medicine and Dentistry.

The work of Cori and others has definitely established the ability of epinephrine materially to increase the blood lactic acid and blood sugar. Cori and Buchwald[1] produced positive increments in blood lactic acid and sugar in man by continuous intravenous injections of epinephrine. Similar results were obtained in rabbits by subcutaneous injections.

Ephedrine has produced results similar to those given by epinephrine. Himwich, *et al.*,[2] and Nitzescu and Munteanu[3] report work upon amytalized dogs in whom they obtained increases in blood lactic acid after ephedrine injections of 40-50 mg. per kilo. Wilson[4] found a hyperglycemia in dogs and rabbits after the subcutaneous injection of ephedrine in doses of 10-30 mg. per kilo.

The experiments herein reported were performed upon normal students. Ephedrine sulfate was ingested in aqueous solution in doses of 60-90 mg. Venous blood samples were drawn at intervals without stasis and the blood lactic acid determined by the method of Friedemann and Kendall.[5] Blood sugar was determined by Benedict's method. (Table I.)

TABLE I.

	Subject E.D.		Subject G.D.		Subject G.S.C.		Subject G.D. (Epinephrine)		
Time	mg.% Lactic Acid	mg.% Blood Sugar	mg.% Lactic Acid	mg.% Blood Sugar	mg.% Lactic Acid	mg.% Blood Sugar	mg.% Lactic Acid	mg.% Blood Sugar	Time for Epinephrine
Normal	20.8	98.8	29.2	117.5	23.3	77.7	13.8	102.2	Normal
45 min.	27.3	106.0	29.7	125.0	21.7	84.1	23.0	102.2	15 min.
75 "	35.2	106.5	38.0	112.0	18.8	100.7	30.7	131.5	30 "
105 "	18.8	106.5	83.6	123.0	29.6	112.5	34.0	148.1	45 "
145 "			21.4	114.0	18.8	110.2	30.0	129.0	75 "

[1] Cori and Buchwald, *Am. J. Physiol.*, 1930, **45**, 71.

[2] Himwich, *et al.*, *J. Biol. Chem.*, 1930, **85**, 571; PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 331; *Ibid.*, 1930, **28**, 333.

[3] Nitzescu and Munteanu, *Comp. Rend. des Seances de la Soc. de Biol.*, 1931, **106**, 1173.

[4] Wilson, J. Allen, *J. Pharm. Exp. Therap.*, 1926, **30**, 209.

[5] Friedemann and Kendall, *J. Biol. Chem.*, 1929, **82**, 23.

In view of the calorigenic action of epinephrine and the similar effects of epinephrine and ephedrine upon blood lactic acid, it was decided to study the effect of ephedrine upon the respiratory metabolism. The Benedict-Universal metabolism machine was used in following the changes in oxygen consumption and CO_2 output after ephedrine ingestion. The metabolic rate was determined in the post-absorptive or "basal" state after at least 30 minutes rest in bed. Immediately after the basal run a blood sample was taken for lactic acid analysis. Ephedrine, in doses of 1 mg. per kilo was then ingested and after the effects had become pronounced (as indicated by heart rate) the metabolism was again determined. Positive increments of blood lactic acid and oxygen consumption were obtained. (See Table II.)

TABLE II.

Subject	Normal				After 1 mg. Ephedrine per kilo			
	liters O_2 / HR	liters CO_2 / HR	R.Q.	mg. Lac. Acid	liters O_2 / HR	liters CO_2 / HR	R.Q.	mg. Lac. Acid
L. C. M.	13.35	11.03	.825	13	14.20	13.35	.94	29
K. G. M.	13.39	11.40	.82	24	15.80	13.90	.88	40.5
K. G. M	11.50	11.40	.99	12.8	9.76	12.10	1.24	28.5
G. S. C.	11.70	11.65	.995	29.2	8.87	13.70	1.55	49.5
G. D.	14.79	12.78	.81		16.37	12.0	.95	
E. D.	13.13	9.83	.749		13.80	14.46	1.06	
L. C. M.	15.70	12.82	.815		16.20	14.6	.90	

Tainter[6] classifies ephedrine pharmacologically with tyramine and phenylaminoethanol, both musculotropic in action, and not with epinephrine, a sympathomimetic drug. He bases his classification upon the antagonism of cocaine towards ephedrine as compared to sensitization of epinephrine action under similar conditions. De Eds and Butt[7] could not obtain the well-known "reversal" phenomenon in the uterine muscle preparation after ergotoxine and ergotamine, when using ephedrine.

On the other hand, Chen and Schmidt,[8] Curtis,[9] and others maintain ephedrine to be sympathomimetic, perhaps not exclusively, but predominantly. Curtis showed that De Eds had used too large a dosage of ephedrine to produce the reversal action on the isolated uterus. He obtained reversal actions with both ephedrine and epinephrine in equi-molecular quantities. Swanson[10] studied the effects

[6] Tainter, M. L., *J. Pharm. Exp. Therap.*, 1929, **36**, 569.

[7] De Eds and Butt, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 550.

[8] Chen and Schmidt, Ephedrine and Related Compounds. Monograph.

[9] Curtis, *J. Phar. Exp. Therap.*, 1928, **34**.

[10] Swanson, E. E., *J. Pharm. Exp. Therap.*, 1929, **36**, 541.

of ephedrine and epinephrine on the bronchioles in cocainized and normal animals. He concluded that ephedrine is both bronchoneurotropic and bronchomusculotropic.

Epinephrine, admittedly a sympathomimetic drug, produces an increase in blood lactic acid, blood sugar, and oxygen consumption. Ephedrine, whose mode of action is debated, produces effects comparable to those of epinephrine in (1) increasing the blood lactic acid and blood sugar, and (2) increasing the oxygen consumption and metabolic rate, hence is it not reasonable to assume that its action in these instances at least, is sympathomimetic?*

6113

Production of Renal Insufficiency by Surgical Procedure.

DOUGLAS R. DRURY.

From Collis P. Huntington Memorial Hospital, Boston.

This communication describes a method which has been developed to produce renal insufficiency by surgical procedure. Rabbits were used. The method is carried out by putting a loop of thread about one renal artery, when the animal is about 10 days old. This is done by placing a wire of 0.4 mm. diameter alongside the artery and tying a silk thread down upon the 2 together, and then withdrawing the wire. This leaves a loop about the artery of a diameter little larger than the artery. The artery soon grows up to this size and then any increase in the blood flow and size of that kidney is prevented. The other kidney overdevelops and so takes care of the increased renal demands of the animal. When the animal is 3 to 4 months of age, this large kidney is removed, leaving the animal with a small kidney, which cannot hypertrophy because of the tie about the artery. Too much time must not elapse before the second operation, lest atrophy of the small kidney supervene.

In this way any degree of kidney insufficiency can be produced. If the reduction in renal tissue be large (four-fifths) we find a rise in blood non protein nitrogen to 50-80 mg. per 100 cc. of blood, and a large volume of dilute urine is excreted. Greater reduction in kidney tissue results in higher blood non protein nitrogen (120-150

* The author is deeply appreciative for the helpful advice and criticism of Dr. R. G. Sinclair and for the cooperation and advice of Dr. E. E. Hawley of the Department of Vital Economics.

mg. N per 100 cc.), symptoms like uremia, low plasma chlorides, and death.

6114

Disturbance of Carbohydrate Metabolism in Normal Dogs Injected with the Hypophyseal Growth Hormone.*

HERBERT M. EVANS, KARL MEYER, MIRIAM E. SIMPSON AND FREDERICK L. REICHERT.

From the Institute of Experimental Biology, University of California, and the Halsted Surgical Laboratory, Stanford University.

Clinical and experimental data have been accumulating indicating a functional relationship between the anterior lobe of the hypophysis and the pancreatic islets. (See especially Houssay *et al.*) Due to the importance of such a relationship we think it justifiable to note briefly some remarkable findings bearing on this matter.

Two litters of purebred dachshund, one consisting of 2 males, the other of 2 females were secured. One male and one female were injected daily intraperitoneally with the anterior hypophyseal growth hormone,† free of gonad stimulating hormone, for a period of about 8 months. Skeletal growth and body weight greatly exceeded that of the litter-mate controls, the weight of the injected animals soon being double that of their controls. Skin overgrowth and folding were present in both but was particularly prominent in the male. After 8 months of daily injection the male developed polydypsia, polyuria, polyphagia and became emaciated. The animal suffered from skin infection with loss of hair. He was inactive and evidently sick. Fehling's test of the urine was strongly positive. Only a trace of albumin was present in the urine. Fasting blood sugar was 232 mg. %. When injection was stopped for one week the animal improved, but rapidly failed again on resumption of injection. At present, 4 months after cessation of injection, the animal still has sugar in the urine. The test for albumin is now negative. The volume of urine has decreased and the animal has almost completely recovered.

* Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of the University of California.

† This was a further purified solution made from the acetone precipitate of the Evans-Simpson-Cornish extract. See Evans, Simpson and Cornish, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 101.

The female dachshund has now been injected one year. Though responding markedly to the growth hormone she has never shown clinical or laboratory evidence of disturbance of carbohydrate metabolism. Urine and blood sugar have remained normal.

From another litter of dogs, of shepherd breed, one female and one male were injected chronically with the same purified growth hormone. The skeletal growth in these dogs was not markedly greater than that of their control, but weight greatly increased. Skin folding was prominent in the female. After about 9 months of injection the female began excreting sugar without being conspicuously sick. She suffered from skin infections. The blood sugar was 228 mg. %. Albumin was present in the urine in a trace or was absent. When injections were stopped she became sugar-free in 2 months. The male litter-mate is still being injected after one year and though obese he is still well and the urine has always been sugar-free.

The absence (or traces only) of albumin in the urine seems to rule out general amyloid degeneration as the cause of the disturbance in sugar metabolism. The evidence indicates that the interrelation of pancreatic islets and anterior pituitary is mediated by the growth hormone and not by the gonad-stimulating principle. Insulin and the growth hormone are thus antagonists, not synergists. It is remarkable that the insular mechanism of normal animals may be injured by simple conveyance of large amounts of the growth hormone. The "constitutional" differences of animals are evident in the differing response of litter-mates to *identical* treatment for, as explained, litter-mates may show no disturbance of carbohydrate metabolism though handled concurrently and identically.

6115

Demonstration of the Anti-Anemic Factor in Bovine Gastric Juice.

HARLEY A. WILLIAMS AND JOSEPH B. VANDER VEER.
(Introduced by J. T. Wearn.)

From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland.

Following the observation of Castle and his coworkers[1, 2] that normal human gastric juice activates beef to produce an anti-

[1] Castle, W. B., *Am. J. Med. Sci.*, 1929, **178**, 764.

[2] Castle, W. B., and Townsend, W. C., *Am. J. Med. Sci.*, 1929, **178**, 764.

anemic substance, it seemed worthwhile to test bovine gastric juice for this property. The present report deals with a study that is similar to those of Castle but different in that bovine gastric juice was used instead of the stomach contents from human beings.

We obtained fresh gastric juice from the fourth stomach pouch of adult cattle. This material was filtered until a water-clear product was obtained and then incubated with beef at 37°C. for 2 hours. The incubated product was strained and portions administered by stomach tube to 4 patients having classical pernicious anemia.

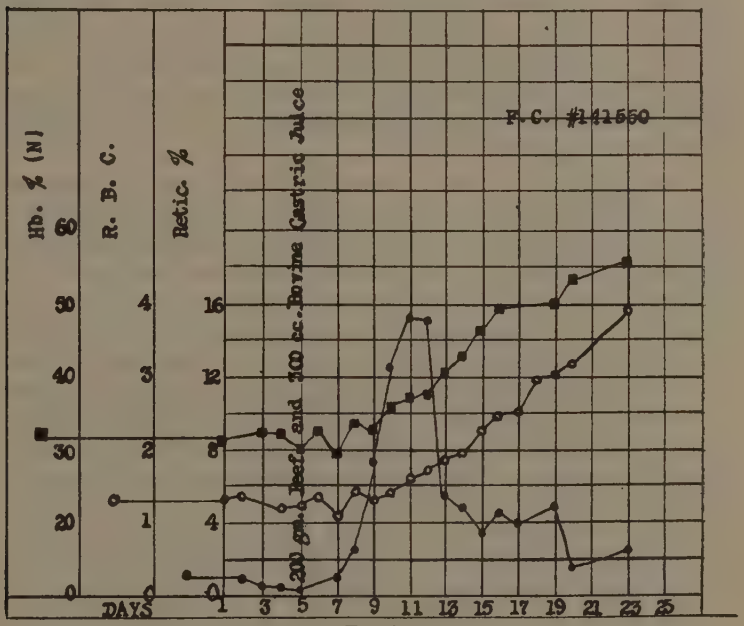

FIG. 1.

The diagram shows a characteristic remission of pernicious anemia (Case 1). The reticulocytes, red blood cells, and the hemoglobin all behaved in the manner described by Minot.[3]

These experiments demonstrate conclusively that the unpurified gastric juice taken from the fourth stomach pouch in cattle contains a potent anti-anemic factor. This work closely parallels the work of Castle and, in addition, makes it possible for us to continue studies without resorting to human sources for gastric juice.

[3] Minot, G. R., Murphy, W. P., and Stetson, R. P., *Am. J. Med. Sci.*, 1928, **175**, 581.

6116

Secondary Calcium Phosphate Prevents and Cures Rickets Without Vitamin D. 1. Utilization Studies.

CHARLES JAMES BLOOM. (Introduced by Charles W. Duval.)

From the Graduate School of Medicine, Tulane University of Louisiana.

These studies were undertaken (1) to demonstrate the capacity of the animal organism to utilize the calcium contained in these compounds as compared with the utilization of calcium from other sources. (2) To compare bone-forming properties of refined "pure" dicalcium phosphate,† unrefined dicalcium phosphate,‡ and other calcium compounds. (3) To determine the availability to the organism of various sources of calcium after their incorporation with alimentary fluids.

To determine the relative efficiency of different calcium compounds, feeding experiments were conducted with the albino rat.

When calcium is provided not to include phosphorus, *i. e.*, the carbonate or citrate, the retention is seriously interfered with. Whatever phosphorus is supplied as a constituent of the other ingredients of the diet, is not satisfactorily utilized. On the other hand, when the calcium is supplied as calcium phosphate, the phosphorus retention is greatly improved. In spite of this, however, the efficiency with which the calcium is utilized appears to be more dependent on the form of the calcium phosphate. The average calcium retention observed during the periods of feeding tricalcium phosphate was 30.9%, the retention values observed in the unrefined dicalcium phosphate and refined dicalcium phosphate periods averaged 46.8% and 41.2%, respectively. The superiority of the latter substances to tricalcium phosphate probably is related to their greater availability, *i. e.*, solubility in the digestive fluids.

Conclusions. 1. The calcium contained in unrefined and refined dicalcium phosphate is utilized much more efficiently than are equivalent quantities of calcium supplied in the form of carbonate or citrate. 2. That this is not due solely to the fact that phosphorus is a limiting factor in these diets is demonstrated by the fact that the calcium of refined dicalcium phosphate is utilized about 70% more efficiently than is the calcium in tricalcium phosphate. 3. Unless adequate quantities of phosphorus, as well as of calcium, are assured, progressive changes take place resulting in the decalcification

† Dicalfos, manufactured by the Bay Chemical Co., New Orleans, La.

‡ Dicapho, manufactured by the Bay Chemical Co., New Orleans, La.

TABLE I.
Showing Percentage Composition of Diets Used in Refined and Unrefined Dicalcium Phosphate Metabolism Studies.*

Diet No.	150	151	152	155	156
Wheat Gluten	20.0	20.0	20.0	20.0	20.0
Sodium Chloride	1.0	1.0	1.0	1.0	1.0
Yellow Corn	77.0	75.56	75.56	74.5	76.68
Calcium Carbonate	2.0	—	—	—	—
Calcium Citrate	—	—	—	4.5	—
Tricalcium Phosphate	—	—	—	—	2.32
Dicalcium Phosphate (unrefined)	—	—	3.44	—	—
Dicalcium Phosphate (refined)	—	3.44	—	—	—
Calcium	1.065	0.903	0.944	0.911	0.673
Phosphorus	0.307	0.833	0.922	0.307	0.653
Ca : P	3.47	1.02	1.02	2.97	1.03

*This diet differed from the Steenbock diet only in that only 2% of calcium carbonate was present rather than 3%, the difference being made up in yellow corn. This change was made so as to avoid extreme bone pathology during the course of the experiments such as would have resulted from the higher calcium:phosphorus ratio.

of the bones, even to the extent of actual losses of calcium from the body. 4. The greater efficiency with which calcium is utilized when provided in the form of unrefined and refined dicalcium phosphate is demonstrated roentgenographically by the extent of calcification of the leg bones. 5. The efficiency with which the phosphorus content of the diet is utilized is dependent upon the form in which calcium is supplied. /6. In respect to the efficiency with which the bone-forming elements, calcium and phosphorus, are utilized, unrefined dicalcium phosphate and refined dicalcium phosphate rank superior to tricalcium phosphate or bone-meal, and the latter in turn ranks above the non-phosphorus-containing salts, calcium carbonate and citrate.

6117

Secondary Calcium Phosphate Prevents and Cures Rickets Without Vitamin D. 2. Calcification Studies.

CHARLES JAMES BLOOM. (Introduced by Charles W. Duval.)

From the Graduate School of Medicine, Tulane University of Louisiana.

The preventive and curative methods for experimental rickets were employed. The standard rickets-producing diet had the following composition: Yellow Corn, 76%; Wheat Gluten, 20%; Calcium Carbonate (precipitated chalk), 3%; Sodium Chloride, 1%. Since the precipitated chalk was not 100% calcium carbonate, the resultant calcium content of this diet was 1.13%.

Corresponding diets were made up containing refined, *i. e.*, pure, dicalcium phosphate* and unrefined dicalcium phosphate† in place of the calcium carbonate, the amounts being 4.54% and 4.25%, respectively. These diets provided the same calcium content (1.13%) as the calcium carbonate diet. The above figures were derived from the fact that the calcium content of the various salts, as determined by analysis, were: Precipitated Chalk, 36.69%; Refined Dicalcium Phosphate, 24.81%; Unrefined Dicalcium Phosphate, 26.56%.

The higher proportion of inorganic salt introduced by refined or unrefined dicalcium phosphate was compensated by a corresponding reduction of corn content.

Healthy albino rats were divided into 5 groups:

Group A received the calcium carbonate diet only, for a period of 35 days.

Group B received the refined dicalcium phosphate diet only, for a similar period.

Group C received the unrefined dicalcium phosphate diet only, for a similar period.

Group D received the carbonate diet for a period of 3 weeks and was then put on the refined dicalcium phosphate diet for 5 succeeding weeks.

Group E received the carbonate diet for 3 weeks and was then changed to the unrefined dicalcium diet for 5 weeks.

The bones of the animals in Group A, which showed +++ or ++++ rickets roentgenographically, yielded an average ash value of 30.69%, which is characteristic of rickets. On the other hand, the groups of animals receiving their calcium in the form of refined dicalcium phosphate and unrefined dicalcium phosphate showed average bone ash values of 50.27% and 51.16%, respectively. These figures corroborate the state of calcification shown by the roentgenographs.

The groups of animals which were allowed to develop rickets and were later cured by substituting for the calcium carbonate, refined dicalcium phosphate and unrefined dicalcium phosphate gave average bone ash values of 49.19% and 46.33%, respectively. These values are normal and are substantiated by the roentgenographs.

Conclusions. The substitution of refined dicalcium phosphate or unrefined dicalcium phosphate for calcium carbonate in the rickets-producing diet resulted in the prevention and cure of rickets. Dis-

* Dicalfos, manufactured by the Bay Chemical Co., New Orleans, La.

† Dicapho, manufactured by the Bay Chemical Co., New Orleans, La.

TABLE I.
Bone Ash Values of Rats Receiving Calcium Carbonate, Refined and Unrefined Dicalcium Phosphate Diets.

Group	Type of Experiment	Calcium Salt	Bone Ash (Dry, fat-free basis)
			%
A	Preventive	Carbonate	30.33
			28.97
			32.74
			28.42
			33.00
		Average	30.69
B	Preventive	Dicalcium Phosphate (refined)	45.64
			51.79
			53.19
		Average	50.27
C	Preventive	Dicalcium Phosphate (unrefined)	49.68
			52.26
			51.53
		Average	51.16
D	Curative	Dicalcium Phosphate (refined)	47.26
			51.11
		Average	49.19
E	Curative	Dicalcium Phosphate (unrefined)	43.92
			48.74
		Average	46.33

regarding the effect of an external supply of phosphorus in the diet, these experiments indicate that from the standpoint of bone formation, refined dicalcium phosphate or unrefined dicalcium phosphate are infinitely superior to calcium carbonate in its various forms, such as precipitated chalk, crushed limestone, or crushed shells. Hence it is proper to refer to refined and unrefined dicalcium phosphate as being indicated in cases of rickets, or leg weakness of chicks.

6118

Secondary Calcium Phosphate Prevents and Cures Rickets Without Vitamin D. 3. Solubility Studies.

CHARLES JAMES BLOOM. (Introduced by Charles W. Duval.)

From the Graduate School of Medicine, Tulane University of Louisiana.

In the experiments here reported, artificial digestion of calcium salts was carried out by shaking an excess of the respective salts with water or hydrochloric acid of 0.1, 0.2, and 0.4% strength. Ten grams of the calcium salt were digested for 24 hours in 100 cc. of the solvent at a temperature of 38°C. The strength of acid and the temperature thus simulated conditions encountered in the animal organism. The suspensions were shaken at 15-minute intervals during the day, but remained undisturbed over night. That this procedure resulted in complete saturation was demonstrated by the repetition of some of the experiments, employing a shaking machine contained in an air thermostat adjusted at 38°C., which corroborated the solubility figures to be reported. At the completion of the digestion the suspensions were rapidly centrifuged and the clear supernatant liquids analyzed for calcium and phosphorus.

In so far as the solubility of these salts in the acid medium of the stomach is concerned, the results indicate that calcium in the form of unrefined dicalcium phosphate* is from 1.7 to 2.7 times as avail-

TABLE I.
Available Calcium in Solutions of Different Salts in Hydrochloric Acid and Water at 38°C.

Solvent	Salt	Cal. per 100 cc. Filtrate
		mg.
Water	Dicalcium Phosphate (unrefined)	39.5
	Dicalcium Phosphate (refined)	15.1
	Tricalcium Phosphate	15.8
	Bone Meal	8.0
0.1% Hydrochloric Acid	Dicalcium Phosphate (unrefined)	154.8
	Dicalcium Phosphate (refined)	131.6
	Tricalcium Phosphate	68.3
	Bone Meal	56.7
0.2% Hydrochloric Acid	Dicalcium Phosphate (unrefined)	266.6
	Dicalcium Phosphate (refined)	247.2
	Tricalcium Phosphate	119.6
	Bone Meal	130.3
0.4% Hydrochloric Acid	Dicalcium Phosphate (unrefined)	503.0
	Dicalcium Phosphate (refined)	484.7
	Tricalcium Phosphate	252.0
	Bone Meal	290.4

* Dicapho, manufactured by the Bay Chemical Co., New Orleans, La.

TABLE II.
Available Phosphorus in Solution of Different Salts in Hydrochloric Acid and Water at 38°C.

Solvent	Salt	Phos. per 100 cc. Filtrate
		mg.
Water	Dicalcium Phosphate (unrefined)	36.0
	Dicalcium Phosphate (refined)	24.6
	Tricalcium Phosphate	55.6
	Bone Meal	1.9
0.1% Hydrochloric Acid	Dicalcium Phosphate (unrefined)	104.8
	Dicalcium Phosphate (refined)	93.9
	Tricalcium Phosphate	55.3
	Bone Meal	13.2
0.2% Hydrochloric Acid	Dicalcium Phosphate (unrefined)	187.3
	Dicalcium Phosphate (refined)	183.0
	Tricalcium Phosphate	60.3
	Bone Meal	37.1
0.4% Hydrochloric Acid	Dicalcium Phosphate (unrefined)	354.8
	Dicalcium Phosphate (refined)	340.8
	Tricalcium Phosphate	82.5
	Bone Meal	100.9

able as the calcium in bone-meal; and that the phosphorus in unrefined dicalcium phosphate is from 3.5 to 8.0 times as available as the phosphorus in bone meal. Refined dicalcium phosphate† is slightly less soluble in the acid media than is unrefined dicalcium phosphate. The tricalcium phosphate used in these experiments showed better solubility than bone meal in the weaker dilutions of hydrochloric acid, but this superiority diminished with increasing strength of acid.

Conclusions. From the standpoint of solubility in hydrochloric acid solutions of the concentration and at the temperature of gastric juice, unrefined dicalcium phosphate is greatly superior to bone meal. The calcium and phosphorus of unrefined dicalcium phosphate are also more available than these elements in the form of tricalcium phosphate. Refined dicalcium phosphate bears a similar relation to these salts as unrefined dicalcium phosphate does, although the solubility figures for the latter are slightly higher.

† Dicalfos, manufactured by the Bay Chemical Co., New Orleans, La.

6119

Presence of a Specific Toxic Factor in the Stools and Urines of Poliomyelitis Patients.

JOHN A. TOOMEY. (Introduced by Victor C. Myers.)

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

The subcutaneous injection of urine specimens or saline emulsions of stools obtained from patients ill with poliomyelitis brought about the death of guinea pigs in greater numbers than similar specimens obtained from normal individuals.

Of 201 guinea pigs injected with stool suspensions from infantile paralysis patients, 66% died and 95% had reactions ranging from a simple local induration to a generalized cystic condition of the abdomen.

Of 120 guinea pigs injected with stool suspensions from normal adults, 41% died and 60% had local or generalized skin reactions.

Of 42 guinea pigs injected with stool suspensions from normal infants, 3% died and 21% had local or generalized skin reactions.

Of 145 guinea pigs injected with suspensions of stools from patients ill with diseases other than infantile paralysis, 23% died and 50% had local or generalized skin reactions.

Of 214 guinea pigs injected subcutaneously with urines from infantile paralysis patients, 14% died and 61% had local or generalized skin reactions.

Of 51 guinea pigs injected with urines from normal patients, none died and but 5% showed local reactions.

Of 139 guinea pigs injected with urines of patients with diseases other than poliomyelitis, 7% died and 12% had local reactions.

Convalescent poliomyelitis serum previously injected intraperitoneally into guinea pigs prolonged their lives, prevented massive local reactions or even protected them from death when later they were injected with stool emulsions or urine specimens from poliomyelitis patients.

That there was a specific toxic factor in the stools and urines of poliomyelitis patients was evidenced by the fact that serum taken from patients at the height of the disease (51 out of 87 cases) did not protect, while convalescent serum taken from the same patients some 21 days after the infection had started, protected guinea pigs which were later injected with stool suspensions or urine specimens obtained from poliomyelitis patients.

The acute and convalescent sera of the remaining patients were about equal in protective value. They were, however, obtained from patients admitted to the hospital usually some time after the disease had started so that the admission blood serum was actually a convalescent poliomyelitis serum specimen.

Recently, it has been shown that dialyzed concentrate of the feces might produce poliomyelitis in *Macacus cynomolgus*.[1]

6120

Increased Production of Colon Agglutinins in Blood Sera of Infantile Paralysis Patients.

JOHN A. TOOMEY. (Introduced by Victor C. Myers.)

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

The colon bacillus was not the prime factor causing the death of guinea pigs after subcutaneous injections of standardized emulsions of stools from infantile paralysis patients. Nevertheless, this organism might have some part in the disease picture since convalescent serum protected coli injected guinea pigs to a slight extent, prolonging the lives of the animals, though not ultimately saving them. This slight protection might be due to an antibody in the serum, perhaps, an agglutinin.

Twenty-three coli-paratyphoid organisms, obtained partly from the American Type Culture Collection and partly isolated by us were used to determine the relative agglutinating capacity of serum obtained at the height of the disease and convalescent serum obtained 21 days after the onset of the disease. Both specimens were taken from the same patient.

Of 88 cases whose sera were thus tested, 55 showed marked increases in the agglutination titer of the convalescent sera as compared to the sera obtained in the acute stages. Thirty-one cases had sera of equal agglutination titer, while 2 cases had higher titers with the acute serum than with the convalescent serum.

[1] Clark, P. F., Roberts, D. J., and Preston, W. S., Jr., *J. Prev. Med.*, 1932, **6**, 47.

6121

Reactions of Poliomyelitis Patients to Autonomic Drugs.

JOHN A. TOOMEY. (Introduced by Victor C. Myers.)

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

In infantile paralysis, the superficial abdominal reflex is lost or modified in whole or in part, often before somatic paralysis appears. Theoretically then this loss could not be due to a spinal nerve involvement but might be due to sympathetic nerve paralysis. Since we have believed that the disease is essentially of gastrointestinal origin, the possible connection between the sympathetic system and the gut was given consideration. Experiments with autonomic drugs were performed to show this connection.

In typical cases of the spinal type of the disease, the patients were injected in the paralyzed and non-paralyzed areas with atropine, histamine, adrenalin, etc., without definite results. Salt solution was injected intradermally over the paralyzed areas, but the comparative data were useless.

When pilocarpine was injected subcutaneously, remote from the part paralyzed, the patients began to sweat first over the paralyzed area and only later over the corresponding normal side. A few minutes later the unparalyzed side stopped sweating, but the paralyzed side usually continued to be moist.

Since the sweat glands are solely innervated by the thoracolumbar outflow, their stimulation was then attempted by adrenalin. When adrenalin was given such a patient at the height of the sweating response to pilocarpine injections, the sweating ceased promptly over the unaffected area, but the patient continued to sweat over the paralyzed side, showing that the thoracolumbar sympathetic outflow of that particular segment was paralyzed. Since this reaction occurred often before somatic paralysis, it directed attention to the sympathetic system as the first part of the nervous system to be involved in this disease.

6122

Comparison of the Blood Picture in Treated and Untreated Syphilis Patients.

PAUL D. ROSAHN AND LOUISE PEARCE.

From the Laboratories of The Rockefeller Institute for Medical Research.

Blood examinations were made on a number of syphilitic patients* who were divided into 2 groups. One group consisted of 58 patients who had received no treatment up to the time of the blood examination. Thirty-two were seen in the stage of an active primary infection, 18 had active secondary lesions, and 8 had signs and symptoms of tertiary disease. The second group consisted of 55 patients who had received varying amounts of specific treatment, ranging from less than one full course of arsphenamine and mercury to several such courses. In 20 of these, treatment was instituted during the primary stage of the disease, in 16 treatment was begun during the secondary stage, and in 19 during the tertiary stage.

Since the patients were ambulatory and repeated counts could be obtained only with great difficulty, one complete blood examination was made on each individual. Each examination included a total red and white cell count made with standardized pipettes, a hemoglobin determination by the Newcomer method, a platelet count by the Ringer-heparin method of Casey,[1, 2] and a differential white cell count made with the supravital technique, 100 cells being counted on each of 2 smears. The blood findings in these 2 groups were then compared, and the mean and standard error of the mean was determined for each blood element. A difference was considered to be significant when the probability of its occurrence by chance was less than one in 100.

In the treated group as compared with the untreated patients, the hemoglobin level and the absolute and relative numbers of lymphocytes were significantly higher, while the total white cells, the number of platelets, the absolute and relative numbers of neutrophiles, and the absolute and relative numbers of monocytes were significantly lower. The values for these blood elements in the treated cases approached the assumed normal values for normal

* These patients were made available for study through the kind cooperation of Dr. Howard Fox.

[1] Casey, A. E., and Helmer, O. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 655.

[2] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 523.

individuals. The erythrocyte count was higher in the treated group. Although this change was not significant, the increase in the number of red cells was reflected by a significantly higher hemoglobin value in this group. In absolute numbers, basophiles were higher and eosinophiles lower in the treated cases, but because comparatively few of these cells were present, these differences were not statistically significant.

The monocyte-lymphocyte ratio, which has been suggested by Cunningham and Sabin[3] and their coworkers as a prognostic aid in tuberculosis, is of especial interest. In the untreated group of this study the M/L index was 0.81 while the treated group gave a significantly lower M/L index of 0.52. This difference was due to a higher lymphocyte and a lower monocyte level in the treated group. Eleven treated patients whose serological and spinal fluid examinations were persistently negative, had the lowest M/L index of 0.35. Further observations are being made to determine the significance of the monocyte and lymphocyte values in syphilis.

During the period of active lesions in the experimental disease, Pearce[4] has reported a slight increase in the total white cell count and absolute number of neutrophiles, unchanged or lowered lymphocyte values, and an increase in the absolute number of monocytes. To determine the blood cell levels after complete healing of all lesions, examinations have been made of a group of 19 syphilis rabbits from 6 months to one year after inoculation, when there existed no symptomatic evidence of an infection. The total white cell count, and the absolute numbers of neutrophiles, lymphocytes, and monocytes were well within normal limits. A survey of platelet counts on animals in all stages of infection showed high values during the period of active lesions and normal values when healing had taken place.

The blood picture in the group of treated patients differed significantly from that in the untreated group with respect to white cell count, platelet count, and absolute numbers of neutrophiles, lymphocytes, and monocytes. These changes were in the same direction as those observed in a group of rabbits after the complete subsidence of all lesions. It cannot be stated definitely that the changes observed in the treated group were due solely to treatment. It is possible that the changes might have been due to spontaneous variations occurring during the period required for

[3] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.

[4] Pearce, L., *Proc. VIII Inter. Congress Derm. and Syph.*, Copenhagen, 1930.

treatment, and that these differences would have been present regardless of the institution of treatment. Nevertheless, the cellular reaction, whether due to treatment or to the element of time, was identical with that occurring in rabbits which had developed an active immunity and had effectively suppressed the disease without treatment.

6123

Serum Phosphatase Changes in Calcium Deficiency and in Ammonium Chloride Osteoporosis.

AARON BODANSKY, HENRY L. JAFFE AND J. P. CHANDLER.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

It was shown by Kay[1] that the plasma phosphatase increases in certain clinically observed cases of bone diseases. We have confirmed and extended these observations.[2] In an investigation of experimentally produced bone lesions we have reported the effects of chronic and acute hyperparathyroidism.[3] In view of the many analogies observed in bone resorption, whether produced by parathormone or by other agents, it was desirable to extend our observations to the osteoporoses produced by low-calcium diets and by ammonium chloride administration to animals on low and high calcium diets.

Four litters of dogs were used—3, 6, 8, and 18 months old, respectively. The experiment was continued for about 11 weeks. The animals received a diet of fresh, lean horse meat supplemented with cod liver oil and tomato juice. This is a low-calcium diet. Some of the animals received a calcium supplement (2.5 gm. each of bone meal and calcium lactate per kilo of food) equivalent to between 0.5 and 2.5 gm. of calcium daily. On this diet the controls grew rapidly; the bones were normal upon autopsy. On the low-calcium diet, the growth was equally rapid; the bones, however, were thinned. Ammonium chloride was administered by stomach tube in a 1% solution. At the end of the experiment some dogs received daily as much as 1 gm. per kilo. In the youngest litter bone softening

[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

[2] To be published.

[3] Bodansky, A., and Jaffe, H. L., *Proc. Am. Soc. Biol. Chem.*, Montreal, Canada, April 8-11, *J. Biol. Chem.*, 1931, **92**, xvi.

and deformity resulted on a low-calcium diet; in all dogs administration of ammonium chloride generally caused loss of appetite.

The method for determination of phosphatase has been described.[4, 5] Serum phosphatase rather than plasma phosphatase has been determined in this series of tests to avoid the inhibition of phosphatase activity by oxalate.[5] Phosphatase values are influenced by diet, malnutrition and fasting.[5] Depending on the duration of the fast, plasma phosphatase decreased in fasted dogs, rats and guinea pigs to between one-half and one-quarter of the values found in normal controls. These findings must be considered in the interpretation of results in long experiments in which satisfactory nutrition and growth cannot be maintained.

In the 3-month litter the average initial serum phosphatase was 11.0 units per 100 cc. On an adequate calcium diet final values (11 weeks later) in 2 controls were 7.6 and 6.8; on ammonium chloride treatment (1 puppy) the final value was 14.3. On a low-calcium diet plus ammonium chloride (1 puppy) the final phosphatase value was 14.8 units. Thus, in the 3-month litter, ammonium chloride, on an adequate as well as on calcium-poor diet, in spite of malnutrition, caused an upward trend of serum phosphatase instead of the downward trend observed in normal growing puppies.

In the 6-month litter the initial group average was 7.6 units per 100 cc. of serum (minimum 6.2, maximum 9.7). On the calcium-adequate diet 1 control showed a practically constant serum phosphatase (initial 7.2 units, final 7.8—the usual trend at this age is downward); 2 dogs receiving ammonium chloride showed a decreased serum phosphatase (initial 6.9 and 6.8, final 5.4 and 3.7 units). On the low-calcium diet, however, 1 control showed a rise from the initial 6.2 units to a final value of 9.0 units, and ammonium chloride administration (2 dogs) resulted in a rise to final values of 11.6 and 14.1 units (from initial values of 9.2 and 9.7 respectively). In this litter ammonium chloride did not reverse the usual downward trend of the serum phosphatase when the calcium intake was adequate. On the calcium-poor diet, however, the downward trend of the phosphatase was reversed in spite of some loss of appetite.

The 8-month litter (3 *bull terriers*) (initial phosphatase 3.8 units) showed no marked changes due to ammonium chloride either on the high or low calcium diet.

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[4] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 760.

[5] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 199.

In the 18-month litter the initial group average was 2.4 (minimum 2.3, maximum 2.7). On an adequate diet plus ammonium chloride (2 dogs) the final phosphatase values decreased to 1.5 and 1.8. On a low calcium diet, on the contrary, both with and without ammonium chloride treatment, the downward trend was not shown, the final phosphatase values remaining practically unchanged at 2.6 and 2.5, respectively.

The results of these experiments may be summarized as follows:

In growing animals the serum phosphatase normally decreases with age. On an experimental regime involving loss of appetite, further decrease of serum phosphatase would be expected. Therefore the serum phosphatase rise in the two younger litters is all the more convincing evidence of the greater susceptibility of young animals to the effects of experimental calcium deficiency and ammonium chloride acidosis. In older dogs, ammonium chloride is relatively ineffective in producing decalcification and serum phosphatase changes when an adequate calcium supplement is given.

6124

Pernicious Anemia: Method Whereby Therapeutic Efficacy of Liver and Liver Fractions May be Substantially Increased.

GEORGE B. WALDEN AND G. H. A. CLOWES.

From the Lilly Research Laboratories, Indianapolis.

Following the discovery of Minot and Murphy that raw liver is highly effective in the treatment of pernicious anemia, liver fractionation was undertaken by Cohn, Minot and their associates. The Cohn liver fraction G, in which approximately two-thirds to three-quarters of the potency of the original liver is recovered in 3 to 4% of its weight, has been generally employed in the oral treatment of pernicious anemia. Use of this preparation, involving the consumption of 10 to 14 gm. of material daily, entails some inconvenience to the patient. In attempting to isolate and identify the active principle Cohn, West and others have made very highly concentrated preparations but the procedures employed have not found practical application on account of their complexity and the losses of active principle entailed.

We here describe a method whereby the bulk of material administered orally to pernicious anemia cases to produce a given thera-

peutic effect may be substantially reduced, not by employing fractional methods as heretofore, but by so treating the raw liver or liver fractions (Cohn Fraction G) as to increase their therapeutic efficacy several fold.

These experiments were started immediately after Castle's demonstration that a thermolabile agent derived from the normal human stomach could interact with beef muscle to produce an active product which when administered to pernicious anemia cases induced remission with reticulocyte rise and increase in red cell count, while control pernicious anemia cases receiving normal human gastric juices alone and other control cases receiving beef muscle alone exhibited no such effects. Since fresh untreated muscle exerted no effect whatever, while a given weight of fresh muscle previously treated with normal stomach juice exerted a very substantial effect approximately equal to that exerted by an equal weight of fresh raw liver, it appeared important to determine whether a similar pre-treatment with normal stomach juices or stomach tissues would cause a corresponding rise in the therapeutic efficacy of the already active raw liver or liver fractions.

Fresh hog liver was ground and admixed with ground fresh hog stomachs, with and without the addition of acid, and held for varying periods of time, at varying temperatures, and the resulting products or their extracts evaporated *in vacuo* and defatted or subjected to alcoholic fractionation. Considerable difficulty was experienced in the conduct of these preliminary experiments but certain of the preparations obtained when tested on pernicious anemia cases exhibited definite gains in potency as compared with the original raw liver.

Subsequently preparations were obtained exhibiting a potency at least 3 to 4 times that of the raw liver from which they were derived.

Furthermore, the Cohn Fraction G (Liver Extract No. 343) when similarly treated with fresh ground hog stomachs yielded a product which showed a 3 to 4 fold gain in potency. The tests were conducted on pernicious anemia cases in relapse, the value of a given preparation being determined by the reticulocyte peak, the gain in red corpuscles and the general improvement of the patient as compared with the effect exerted by 225 to 300 gm. of raw liver or 3 to 4 vials of Liver Extract No. 343.

This increase in therapeutic efficacy of raw liver and of the Cohn Fraction G as a result of admixture and interaction with a moderate amount of fresh stomach tissues and products derived therefrom,

can not be explained as a purely additive effect. The clinical results indicate that one-third to one-seventh of a unit dose of liver or liver products treated with one-tenth to one-thirtieth of a unit dose of raw stomach or stomach products will exert an effect equal to that of a unit dose of liver or a unit dose of stomach.

These results find support in the report of Reimann[1] that the efficacy of raw liver in the treatment of pernicious anemia is markedly increased by admixture with normal human gastric juice prior to administration.

We desire to express our indebtedness to Dr. John H. Warvel, to Dr. L. G. Zerfas and Dr. P. J. Fouts, and to Dr. Howard L. Alt for conducting tests on pernicious anemia cases in relapse with the above described materials. The detailed clinical results will be reported in the near future.

6125

Value of Desiccation and Identification of Blood Typing Serums.*

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The accuracy of the determination of the blood group depends essentially upon the sterility of the typing serums and the freedom from formation of precipitates. The usual method of preserving stock typing serums is in a liquid form with the addition of 0.5% phenol. Lattes[1] has shown that serums preserved with phenol as well as many other preservatives, become turbid and form precipitates, and advises the use of sterile serums. The length of time that these aseptically collected serums will remain uncontaminated is variable and indefinite. Prati,[2] Grove and Crum[3] have demonstrated that liquid typing serums are very subject to contamination by a mustard bacillus and thus develop in the contaminated serums a non-specific property of human blood cell agglutination.

Desiccation may offer a desirable means of preservation. The value of desiccation is dependent upon its effect in time on the

[1] Reimann, Von, Dr. F., *Med. Klinik*, June 12, 1931.

* Jr. article No. 94 (n.s.) from the Mich. Agr. Exp. Sta.

[1] Lattes, L., *L'individualite du sang*, Paris, 1929, Masson.

[2] Prati, *Z. f. Immunitatsf*, 1928, **57**, 1.

[3] Grove and Crum, *J. Lab. and Clin. Med.*, 1930, **16**, 259.

agglutinins in the serums. Slides were prepared as follows: Near the end of the slide was placed 0.01 cc. of type II serum, according to the Moss classification, containing 0.5% phenol and allowed to dry as a drop in the open air. Similarly near the other end of the slide 0.01 cc. of type III serum containing 0.5% phenol was placed. Each serum was marked below the drop to identify its group. The slides were divided into 2 lots, one lot being held at room temperature and the other at 8°C. Liquid phenolized typing serums were prepared at the same time for controls. These were tested at intervals.

Rosenthal[4] demonstrated that typing serums could be preserved in a liquid form by staining and that they required no particular aseptic precautions in handling and storing. He[5] furthermore demonstrated that the agglutinating titre of stained serums remained practically the same as his aseptic controls kept at ice box temperature, and even at room temperature the stained serums did not lose their transparency. Recognizing the value of stained typing serums in identification and preservation, similar serums were prepared and dried on slides to determine the added value of desiccation. To each cc. of type II serum was added 0.01 cc. of a 1.0% aqueous solution of neutral acriflavine and 0.01 cc. of a 0.5% aqueous solution of basic fuchsin. To each cc. of type III serum was added 0.02 cc. of a 1.0% aqueous solution of brilliant green. As before, 0.01 cc. of each serum was placed on opposite ends of a microscopic slide and allowed to dry as a drop. The colors of the dyes are the essential means of identification and remain evident after the addition of the cells during the typing of a blood. These slides were divided into 2 groups as before, one group being held at room temperature and the other group at 8°C. As a control, liquid stained serums were prepared.

The condition of the preserved serums was determined at intervals by cross-agglutination with cells of known groups.

To insure the best results in using the desiccated serums the following technic is advised. Two medium-sized drops of blood to be typed are collected and mixed in 2 cc. of physiological salt solution. Approximately 0.01 cc. of this cell-salt-suspension is added to both type II and type III desiccated serums on the slide. A tooth-pick is used to mix, reversing ends for each serum. The slide can be gently rotated to facilitate agglutination. Observations are then made either macroscopically or microscopically.

[4] Rosenthal, L., *J. Lab. and Clin. Med.*, 1931, **16**, 1123.

[5] Rosenthal and Hornick, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 515.

The use of dessication of typing serums makes it unnecessary to use precaution as to sterility in handling and storage and simultaneously eliminates the possibility of precipitate formation, which causes confusion in interpreting results in stored liquid phenolized serums. This method makes it possible for the practicing physician to carry the typing serums in his case for emergencies, and hospital technicians can also rely on more accurate results from these preserved serums.

Slides of phenolized serums have been kept at room temperature and 8°C. without deterioration for 11 months, whereas the liquid phenolized serums give a very confusing precipitate in that time. Desiccated and liquid stained serums have kept perfectly to date or 7 months. This study is being continued to determine the maximum length of time the serums can be desiccated and still give accurate results.

6126

Independence of Ventricular Arhythmia from Insufflations to Coronary Flow in Rabbits.

WILLIAM F. ALLEN.

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In a previous paper[1] it was demonstrated that a left ventricular arhythmia produced from insufflations of benzol in tracheotomized animals was contingent on impulses which descend the median part of the lateral columns of the spinal cord and reach the heart by way of the stellate ganglia. The purpose of this report is to record some observations which suggest that this arhythmia is not dependent on the nutrient supply of the heart. This arhythmia is normally preceded by a moderate rise in carotid pressure, a change which Markwalder and Starling,[2] Anrep and Segall,[3] Hochrein, Keller and Mauke[4] would place first, as a means for producing an increased coronary supply. The arhythmia never appeared when blood pressure was low (40 mm. and below). Double vagotomy in no way delayed the onset or shortened the interval of this arhythmia, except possibly for a short interval immediately after sectioning.

[1] Allen, W. F., *Am. J. Physiol.*, 1931, **96**, 243.

[2] Markwalder and Starling, *J. Physiol.*, 1914, **47**, 275.

[3] Anrep and Segall, *Heart*, 1926, **13**, 239.

[4] Hochrein, Keller und Mancke, *Arch. Exp. Path. u. Pharm.*, 1930, **151**, 146.

Porter,[5] Mass,[6] Wiggers,[7] Anrep and Segall,[3] Greene[8] and others demonstrated that the constrictor fibers for the coronaries run in the vagi and Anrep and Segall[3] have demonstrated that double vagotomy causes a considerable increase in the coronary flow.

If a change in the nutrient supply to the nerve-intact heart is the cause of the arhythmia from insufflations, it should be due to an increase in the coronary flow rather than to a decrease. To demonstrate the effect on the pulse of a brief interval of anemia of the left ventricle, the left interventricular artery and the left ventricular vein* were ligated close to the atrium in a number of animals for several minutes, and in a few animals the right interventricular artery was also ligated. In no instance did these ligations evoke a premature systolic arhythmia. At the time of occlusion of each vessel there was a little drop in blood pressure and usually one or two ectopic beats. Since these beats occurred from similar ligations of any part of the ventricle, they doubtless correspond to the premature beats that Hering[9] and many others have obtained from mechanical and electrical stimulation of various parts of the heart.

To determine whether an increased coronary supply to the ventricles is essential for the production of the premature systoles in the arhythmia obtained from insufflations, the following experiment was done: A rabbit under 75 to 85% surgical anesthesia dosage of sodium barbital was prepared for taking carotid and trachial respiratory tracings. The heart was exposed from the left side and an arhythmia was obtained from benzol insufflation. The right interventricular artery, the left coronary vein and the left interventricular artery were then slit longitudinally for some distance, beginning close to the atria (a procedure which never elicited an arhythmia). This required but a few seconds and within 30 seconds benzol was insufflated into the nostrils in an attempt to produce an arhythmia while all the main arteries to the left ventricle were bleeding pro-

[5] Porter, W. T., *Boston Med. and Sur. J.*, 1896, **134**, 39.

[6] Mass, P., *Pflüger's Arch.*, 1898, **71**, 399.

[7] Wiggers, C. J., *Am. J. Physiol.*, 1909, **33**, 391.

[8] Greene, C. W., *Internat. Physiol. Cong. Abst.*, 1929, **18**, 103.

* A chrome-yellow gelatin injection of the rabbit's heart from the thoracic aorta discloses a different arrangement of the coronary veins than is found in the dog and other laboratory animals. A large left ventricular or great cardiac vein (See Vogt und Yungs' Anatomie) takes origin from the apex of the left ventricle and follows superficially in the ventricle about midway between the 2 interventricular arteries to enter the atrio-ventricular groove and end in the coronary sinus. Several right ventricular veins drain the right ventricle.

[9] Hering, H. E., *Pflüger's Arch.*, 1900, **82**, 1.

fusely. Under these conditions arterial pressure remained above the necessary height for obtaining an arhythmia much longer than was expected from the hemorrhage. The pulsating heart prevented clotting.

Five animals yielded 1 to 3 premature systolic arhythmias following a like number of benzol insufflations at the time the above mentioned coronary arteries were bleeding profusely. A representative record discloses a perfectly regular arhythmia from insufflations in that it is preceded by a moderate rise in blood pressure (16 mm.) and a slowed and strengthened pulse. Every animal which gave the 'insufflation' arhythmia with the heart exposed, produced one from insufflations when the above mentioned coronary vessels were slit. In one animal the arhythmia was obtained after the left interventricular artery and the left ventricular vein had been ligated close to the atrium.

Since the ventricular arhythmia following insufflations of benzol takes place readily under conditions where the blood supply to the ventricles has been greatly reduced and cannot be increased appreciably, when it normally occurs under conditions which should cause a pronounced increase in the coronary intake, suggests that this arhythmia is not contingent on the coronary flow to the ventricles.

6127

On the Property of Certain Normal Animal Sera to Neutralize the Virus of Poliomyelitis.*

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It has been known for a long time that the sera of children and monkeys, who have suffered from poliomyelitis, are capable of neutralizing the specific virus, although the frequency of this occurrence appears by no means to be as regular as was formerly believed[1] More recently a high percentage of normal adults, giving no history of a previous attack or of contact with the disease, have also been found to possess virucidal substances in their blood.[2] A

* Under a grant from the International Committee for the Study of Infantile Paralysis, whose work is being financed by Jeremiah Milbank.

[1] Jungeblut, C. W., and Smith, L. W., *J. Immunol.*, 1932, in press.

number of authors, by indirect inference, have deduced from epidemiological criteria, that this capacity is acquired through exposure to subclinical infections.[3] In contrast to this viewpoint, we[4] have recently presented direct experimental evidence which strongly suggests that resistance to poliomyelitis as expressed by the fall of morbidity and the rising level of serological activity with increasing age, is predominantly a function of normal physiological maturation and to a large extent seems to develop independently of previous contact with the specific antigen. In further support of such a conception—or rather in opposition to the general validity of the exposure theory—the following observations are reported which bear on the presence of virucidal substances in the normal sera of certain susceptible and non-susceptible animals.

Rhesus monkey. There is general agreement in the literature that the serum of *immature* monkeys is uniformly devoid of neutralizing power. We can confirm this point from a summary of 16 tests on a corresponding number of individual samples of serum obtained from normal rhesus monkeys ranging in probable age between 1½ and 3 years. In no case did we observe any neutralization effect with any of the described sera when amounts of 0.6 to 0.8 cc. of serum were tested against 0.6 cc. or 0.4 cc. respectively of a 10% virus suspension (contact 1½ hours at 37°C., overnight icebox).

Quite different results, however, were obtained when normal sera of *adult* rhesus monkeys were studied. These animals were mature or submature when received, judging not only from size but from development of the teeth and sexual organs. The males carried the testicles in the scrotum and the females were passing, more or less regularly, through definite cycles of menstruation. They were kept in separate quarters belonging to the Department of Anatomy and were never in contact with the infected animals nor the attendants handling them. We had an opportunity to test the serum of seven of such animals, one male and six females, for neutralizing power against poliomyelitis virus. Two of these animals were bled twice, so that the samples under investigation numbered nine in all. Four of these nine samples were found to neutralize the virus *in vitro* perfectly, the remaining five exerted no demonstrable virucidal ac-

[2] Shaughnessy, A. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 463. Aycock, W. L., and Kramer, S. D., *J. Prev. Med.*, 1930, **4**, 189 and 201. Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 409. Brodie, M., *J. Bact.*, 1932, **23**, 102.

[3] Aycock, W. L., *J. Am. Med. Assn.*, 1931, **97**, 1199.

[4] Jungeblut, C. W., and Engle, E. T., N. Y. Academy of Medicine, meeting section of pediatrics, Nov. 12, 1931.

tion in the quantities tested. The four neutralizing sera came from four different female animals and were all obtained within 10 days *after* onset of menstrual bleeding. Two of these animals when bled again a few days *before* onset of the next menstrual cycle gave sera which failed to neutralize. We do not know whether this fluctuation in virucidal power of the serum in the same animal is causally related to the various phases of menstruation or has been due to other factors. Work is under way to investigate more precisely the above mentioned possibility in the human. (Table I.)

TABLE I.

Tests for virucidal power of normal adult Rhesus sera.

No.	Sex	Menstruation	Quantities of Serum cc.	Quantities of Virus cc.	Result of Neutralization Test	Control Monkey Paralysis
I	♀	after	0.6	0.6	D76 No paralysis	D57 Complete, 6 days
II	♀	before	0.6	0.6	D87 Partial par., 12 dys.	D78 Almost comp., 12 dys
III	♀	after	0.6	0.6	D53 No paralysis	D57 Complete, 6 days
		before	0.6	0.6	D96 Comp. par., 7 days	D91 " 11 "
IV	♂		0.6	0.6	D97 Comp. par., 9 days	D91 " 11 "
V	♀	after	0.8	0.4	E30 No paralysis	E32 " 12 "
		before	0.8	0.4	E51 Comp. par., 7 days	E48 " 11 "
VI	♀	irreg.	0.8	0.4	E52 Comp. par., 6 days	E48 " " "
VII	♀	after	0.8	0.4	E53 No paralysis	E48 " " "

Technique: serum-virus mixtures incubated 1½ hr. at 37°C. and kept overnight in icebox. 1 cc. of mixture injected intracerebrally.

Cebus monkey. The South American ringtail, or Cebus monkey, is insusceptible to intracerebral inoculation with poliomyelitis virus as has been observed before by Flexner[5] and by Kraus and Kantor.[6] The mechanism of this natural resistance, however, has remained obscure. In this connection it is interesting to point out that the Cebus monkey does not show evidence of a menstrual cycle or periodic bleeding similar to the macaque or human beings. We have attempted to infect four Cebus monkeys by intracerebral inoculation with poliomyelitis virus without producing any symptoms of the disease. Moreover, the animals remained refractory to repeated inoculation after they had been subjected to splenectomy and bilateral castration. Normal sera obtained from these monkeys before inoculation were tested for neutralizing power in the usual manner. Of the 4 sera three neutralized the poliomyelitis virus. The one serum which failed to neutralize was not retested, however the animal proved equally insusceptible to intracerebral inoculation. (Table II.)

[5] Flexner, J., and Lewis, P. A., *J. Am. Med. Assoc.*, 1910, **54**, 45.

[6] Kraus, R., and Kantor, L., *Rev. Inst. Bact.*, Buenos Aires, 1917, **1**, 43.

TABLE II.
Tests for virucidal power of normal Cebus sera.

No.	Sex	Quantities of serum cc.	Quantities of virus cc.	Result of Neutralization Test		Control Monkey		
I	♂	0.6	0.6	Monkey D98	No paralysis	D 91	Comp. paralysis, 11 days	
II	♂	0.6	0.6	" E 7	" "	D100	" " 8	"
III	♂	0.6	0.6	" E50	" "	E 48	" " 11	"
IV	♀	0.6	0.6	" E49	Comp. par., 9 days	E 48	" " 11	"

Technique: same as given for Table I.

Sheep. Neutralizing substances against poliomyelitis virus in normal sheep sera have been encountered occasionally by certain authors (Flexner and Lewis, Stewart and Hasselbauer[7]) while others (Howitt[8]) have recorded only negative results. We have tested a total of four normal sera obtained from as many different adult sheep at various stages of the oestrus cycle.† None of these sera were capable of neutralizing the virus *in vitro,* even when doses as high as 0.8 cc. of serum were run against 0.4 cc. of virus.

Cock. One sample of serum from a cock was tested for neutralization of the virus *in vitro.* The serum failed to neutralize.

The results obtained in this study indicate that absence or presence of the power to neutralize poliomyelitis virus is unevenly distributed over a number of naturally insusceptible animals. As may be gleaned from the literature, a similar irregularity has been noted with normal sera from other insusceptible animals, like the horse, rabbit, and goat, which sometimes neutralize the virus and sometimes fail to do so. Contrasting the previously mentioned animals with the only known species susceptible to experimental infection except the anthropoid apes, *i. e.,* the rhesus monkey, it would seem that neutralizing substances may occasionally be found in the serum of adult animals, while the serum of immature animals reacts uniformly negatively. The close analogy appears obvious between this occurrence and the development of virucidal power with puberty in man. It permits of only one conclusion, in our opinion, namely that normal neutralizing substances, experimentally behaving identically with immune neutralizing substances may occur under conditions which in the present state of our knowledge preclude previous contact with the specific antigen. The relationship of these normal viru-

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.,* 1910, **55**, 662. Stewart, F. W., and Hasselbauer, P., *J. Exp. Med.,* 1928, **48**, 449.

[8] Howitt, B. F., *J. Inf. Dis.,* 1932, **50**, 26.

† These sera were obtained through the courtesy of Dr. F. F. McKenzie of the Missouri Agricultural College, to whom we express our best thanks.

cidal substances to those arising after recovery or active immunization is unsettled and work is in progress to elucidate that point. In this respect the problem is in no way different from the well recognized occurrence in man and animals of other normal 'antibodies' directed against a variety of infectious and non-infectious antigens; and the vexing question as to the precise origin of these agencies in either case remains unsolved, except for the purely empirical observation that physiological maturation is evidently an important governing factor in their development.

Illinois Section.

University of Illinois, April 5, 1932.

6128

The Vagi and Appetite.

P. H. HOLINGER, E. H. KELLEY AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

We desire to record an observation in regard to the relation of the vagus nerves to appetite, an observation indicating that afferent sensations mediated by the vagi may be responsible for anorexia in abnormal gastro-intestinal states. Our reasons for recording an observation on one animal are as follows: (a) The animal preparation on which the observation was made is difficult to care for in that the feeding requires almost the full-time attendance of one individual and there is no likelihood of our being able to repeat the observation on other animals in the near future; and (b) the observation has a direct bearing on the interpretation of the results of those who are now investigating the subject of appetite, especially as related to Vitamin B deficiency, a problem in which we are not interested immediately.

In September, 1930, a dog was prepared[1] with a pouch of the entire stomach with vagi intact and a jejunal fistula for feeding. The pylorus was cut across, the duodenum closed and the pyloric opening brought to the outside, the jejunal fistula having been made previously. Nothing was allowed the dog by mouth for 3 months. At this time we tried to induce the dog to eat, but failed. The dog was on a diet supplemented with vitamines, including Vitamin B concentrate. On rare occasions we were able with much effort to induce the animal to eat an ounce or 2 of food. This continued throughout the period of one year, during which the body weight and nutrition were maintained solely by jejunal feeding. We thought

[1] Scott, Holinger and Ivy, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 569. Scott and Ivy, *Ann. Surg.*, 1931, 1197.

that the appetite mechanism had been "reconditioned", *i. e.*, the dog, "satisfied" with the jejunal method of feeding, had lost the desire to eat by mouth, since the animal was doing well and a desire to take food by mouth could not be stimulated by administration of the various vitamins in large quantities. This explanation, however, was obviously unsatisfactory. Since we were primarily interested in the study of gastric secretion and motility of this animal, we desired to ascertain the effect of sectioning the vagi just above the diaphragm on these processes in this animal preparation. To our surprise, as soon as the animal recovered from the anesthetic, he bolted a pan of food belonging to another dog, and the following day manifested coprophagy. The excessive appetite of the dog has persisted unabated for the past 5 months and might be compared to that of a normal young dog starved for 48 hours. The only interpretation of this sudden return of appetite that has occurred to us is that section of the vagi severed the pathway of the impulses responsible for the production of anorexia. We believe the impulses responsible for the anorexia arose as the result of the disturbed gastro-intestinal state or irritation of the intestine and possibly the colon by the pabulum or method used in feeding. We hope to amplify and extend this observation by other experiments some time in the future.

This observation indicates that the anorexia associated with gastro-intestinal disturbances, and possibly vitamin B deficiency, is due chiefly to afferent sensations mediated by the vagi.

6129

Effect of Reticulo-Endothelial Blockade on Uric Acid of Blood.

R. I. KLEIN AND S. A. LEVINSON.

From the Department of Pathology, University of Illinois College of Medicine.

Folin and his associates[1] demonstrated that uric acid injected intravenously into dogs in standard amounts disappears from the blood in a characteristic manner, *i. e.*, 70% is destroyed within the first 10 minutes and in 2 hours is down to 2 mg. per 100 cc. of blood. They obtained the same results in Eck fistula dogs and concluded that the process occurs within the blood stream, possibly through

[1] Folin, Berglund and Derrick, *J. Biol. Chem.*, 1924, **40**, 361.

some "essential factor" elaborated in the liver. Bollman, Mann and Magath[2] have found that extirpation of the liver in dogs produces an increase in the uric acid of the blood, tissues and urine. When uric acid was injected intravenously into hepatectomized dogs, disappearance was considerably slowed and the uric acid could almost quantitatively be recovered from the urine. It was their opinion that destruction of uric acid in the dog is dependent on the liver.

During studies on the function of the reticulo-endothelial system we observed that when 8% india ink in normal saline was continuously injected intravenously into normal dogs, there was an appreciable increase in the uric acid of the blood. For example, after 6 hours, when 600 cc. of ink had been injected into a 14 kilo animal, the blood uric acid level was 3 mg. per 100 cc. This could be due to an increased production of uric acid or to a diminished destruction. Goldzieher and Sherman[3] observed an increase in urea following ink blockade in mice, rabbits and dogs. They interpreted this as due to an increased activity of the reticulo-endothelial system. We therefore studied the rate of disappearance of intravenously injected uric acid in animals which had been previously blocked.

The uric acid was injected as in the procedure of Folin, Berglund and Derrick, 100 mg. per kilo body weight, *viz.*, a 2% solution with 0.5% lithium carbonate. The blood uric acid was determined by Folin's indirect method.[4]

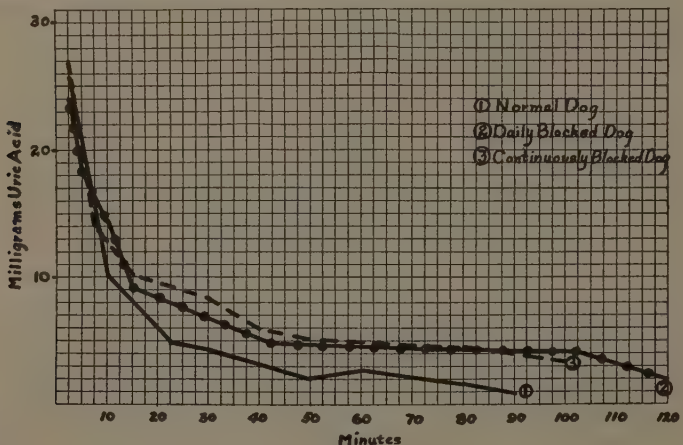

FIG. 1.

[2] Bollman, Mann and Magath, *Am. J. Phys.*, 1925, **72**, 629.

[3] Goldzieher and Sherman, *Arch. Path.* 1931, **12**, 180.

[4] Folin, D., *J. Biol. Chem.*, 1922, **54**, 153.

The chart illustrates the results obtained in typical normal and blocked animals. Curve 1 is that of a normal 9 kilo dog. Two and one-half minutes after the uric acid injection the blood uric acid level was 24 mg. per 100 cc. Within the first 10 minutes it had fallen to 10 mg. and in 90 minutes to 1 mg. This illustrates the normal curve of destruction mentioned previously.[1] Curve 2 is that of an 8 kilo dog that received 4 daily injections of 50 cc. of india ink into a vein of the foreleg. The last ink injection was given the day before the uric acid injection. The uric acid level here parallels that of the normal dog. Curve 3 represents the result obtained in a 14 kilo dog that had just before received a continuous intravenous injection of 400 cc. of ink over a period of 2 hours. This curve also is parallel with that of the normal animals. The slightly higher values at the end of the experiment in the blocked animals is due to the block itself. The control uric acid values after blockade were 0.8 mg. in the daily injected animal and 1.1 mg. in the other.

Saxl and Donath[5] studied the effect of the intravenous administration of electrocollargol on the subsequent injection of water, dyes (phenoltetrachlorphthalein), drugs (adrenalin), and antiseptics (argochrome). Each of these substances was found to remain longer in the circulation than in the control cases without electrocollargol. These findings they attribute to functional paralysis of the reticulo-endothelial system, which normally appears to remove these substances with great rapidity. Whether the reticulo-endothelial cells are stimulated to activity or depressed is, however, not a simple matter, depending on many factors, Jaffe[6] believes.

We believe that by injecting large amounts of material over short lengths of time we obtained good blockade. At autopsy, on gross examination, the liver, spleen, lymph nodes, omentum, peritoneum, adrenals and lungs were all black. The bone marrow seemed resistant. Microscopically the hepatic and splenic endothelial cells were well blocked.

We conclude that blockade of the liver as performed by us does not affect the destruction of uric acid as does extirpation of the liver. The increase of the uric acid in the blood during and immediately after blockade is not apparently due to a defect in uricolysis since intravenously injected uric acid disappears from the blood at a normal rate. The blockade of the reticulo-endothelial system may stimulate the liver cells themselves to an increased production of

[5] Saxl, P., and Donath, F., *Wien. Klin. Wochen.*, 1925, **38**, 66.

[6] Jaffe, R. H., *Physiol. Rev.*, 1931, **11**, 277.

uric acid. It seems, therefore, that the reticulo-endothelial system is not the seat of uric acid destruction in the dog.

6130

Histamine and Acetyl Choline Contraction-Ratio in the Surviving Intestinal Strip.

A. G. WEDUM AND E. GEBAUER-FUELNEGG. (Introduced by A. A. Day.)

From the Departments of Bacteriology and Research Bacteriology, Northwestern University Medical School.

Knowledge of the significance of histamine in biological processes would be materially increased if assays of higher specificity and sensitivity were available. In the absence of such tests it is necessary to look for an increased number of reactions both chemical as well as biological, indicative of histamine. Such reactions might aid by their variety rather than by their specificity.

Experiments with various smooth muscle contractants, forms of shock, etc., now in progress in this laboratory made it advisable to compare the reactivity of chicken and mouse intestine to histamine and acetyl choline. This ratio is well known to be 2:1 in the guinea pig.[1] This figure agrees on the average with our own observations. However, we have not infrequently dealt with individual variations such as guinea pig intestinal strips with a histamine-acetyl choline ratio of 5:1 or 1:1. It should be remembered that the iliac end of the guinea pig intestine is generally conceded to be more reactive than the duodenal.

The action of histamine and acetyl choline upon the mouse and chicken intestine does not appear to have been studied previously. The reactivity of the mouse uterus to histamine has been shown to vary with the concentration.[2, 3, 4] Low concentrations (1:1,170,000) stimulate contraction, while high ones (1:1,250) inhibit. Generally speaking, the use of intestinal strips in preference to other smooth muscle seems advantageous in some instances, because a number of strips of the same intestine can be used and checks can be run conveniently.

[1] Guggenheim, M., *Die Biogenen Amine*, Springer, Berlin, 1924, 103, 219.
[2] Cow, D., *J. Pharm. Exp. Therap.*, 1920, **14**, 275.
[3] Abel, J. J., and Macht, D. I., *J. Pharm. Exp. Therap.*, 1920, **14**, 279.
[4] Adler, L., *Arch. f. Exp. Path. u. Pharm.*, 1918, **83**, 248.

In the present determinations the Schultz-Dale technique was used. The bases were added to the isolated intestinal strip suspended in 25 cc. oxygenated Tyrode's kept at 38-40°C. Dilutions neutralized to phenolphthalein were made in Tyrode's solution so that 1 cc. contained a unit amount of histamine di-hydrochloride* or acetyl choline hydrobromide.† Absence of hydrolysis of the acetyl choline was insured.

In the mouse, chicken, and guinea pig, mixtures of histamine and acetyl choline had an effect equal to the sum of the 2 separate effects, without gross augmentation or retardation.

Sixty-six intestinal strips from 26 white mice showed that the response to acetyl choline was rather regular regardless of sex or weight. Added to the 25 cc. bath, 0.001 mg. of acetyl choline (1-26,000,000 dilution) always caused a decided contraction unless the strip had been weakened by previous tests with other substances. A response to 0.0001 mg. was not infrequent, and in a few instances 0.000,01 mg. caused a contraction. (See Figs. 1, 2.)

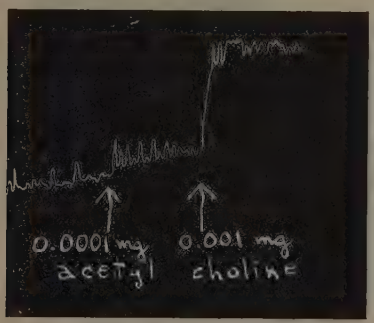

FIG. 1. Mouse Intestine.

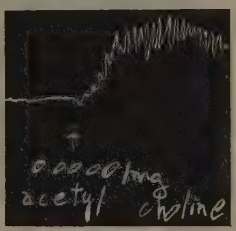

FIG. 2. Mouse Intestine.

The reaction of mouse intestine to histamine was varied. In all amounts less than 50 mg. the histamine usually had no effect. In such an event acetyl choline was added to show reactivity. Sometimes there was a slight alteration of rhythm or a slight relaxation. In 40 strips tested with amounts of histamine varying from 0.0001 to 50 mg., slight contractions were infrequently caused by 0.1 mg., 1 mg., 5.0 mg., and invariably (in each single test on each of 3 mice) by 50 mg. (Fig. 3.) It was concluded that histamine, at least in low concentrations, acted so unreliably that it could not safely be made use of in establishing a ratio.

* Hoffmann LaRoche, c. p.

† Eastman, c. p.

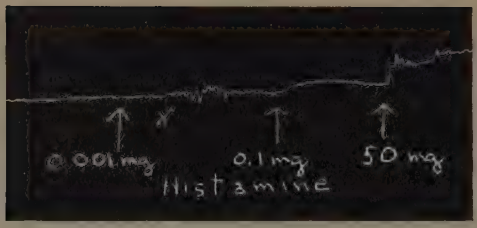

FIG. 3. Mouse Intestine.

Examination of the intestines of 5 chickens one to 3 months old showed that 0.1 mg. histamine or 0.001 mg. acetyl choline certainly caused a contraction, 0.05 mg. histamine often did so, and 0.01 mg. histamine or 0.0001 mg. acetyl choline was sometimes sufficient. (Figs. 4, 5.) This established a ratio of 100:1, which held up very

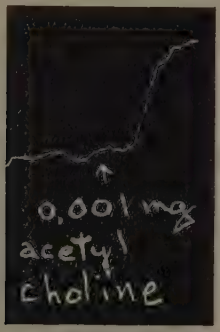

FIG. 4. Chicken Intestine.

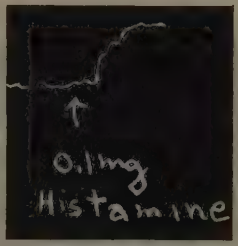

FIG. 5. Chicken Intestine.

well in 4 chickens. However, one of the 5 chickens, obviously ill, had a ratio of 10:1 (0.1 mg. to 0.01 or 0.5 to 0.05).

Naturally, the accuracy of the ratios described is limited by the nature of the dilutions employed, which were multiples of 10. Since individual variations of the ratio are to be found in the test animals described above, such ratios should be regarded as only of suggestive value in assaying the bases as stated in the first paragraph.

6131

Demonstration of an "Anaphylactic Poison".

CARL A. DRAGSTEDT AND ERICH GEBAUER-FUELNEGG.

From the Department of Physiology and Pharmacology, and the Department of Research Bacteriology, Northwestern University Medical School.

That the liver is practically indispensable for the development of anaphylactic shock in the dog has been indicated, principally by Manwaring and his colleagues,[1] Voegtlin and Bernheim,[2] and Simonds and Brandes.[3] Manwaring,[4] in addition, has reported that during anaphylactic shock in the dog, physiologically active substances, called by him "hepatic anaphylatoxins," appear in the circulating blood so that by appropriate cross circulation experiments their presence can be demonstrated by the effects they produce in the recipient animal. A number of considerations led us to suspect that if transportable physiologically active substances are set free during anaphylactic shock in the dog, they might appear in the thoracic duct lymph. Petersen and Levinson[5] have noted the marked increase in the rate of flow of the thoracic duct lymph during shock. That this increase in flow is secondary to the shock phenomena occurring in the liver is indicated by the work of Simonds and Brandes,[6] who reproduced similar effects by mechanical obstruction of the hepatic veins. The points of similarity between the manifestations of anaphylactic shock and mechanical obstruction of the hepatic veins in the dog have been studied in considerable detail by Simonds and Brandes, and warrant the conclusion that a considerable proportion of the increased lymph flow occurring during shock is of hepatic origin. If this is the case, it would seem possible that substances such as the hepatic anaphylatoxins of Manwaring might appear in the lymph.

As one of the most delicate methods of testing for the presence of certain smooth muscle stimulating substances, the isolated surviving strip of guinea pig intestine was used. This method has a number of advantages over cross circulation experiments such as,

[1] Manwaring, W. H., *Johns Hopkins Hosp. Bull.*, 1910, **21**, 275.

[2] Voegtlin, C., and Bernheim, B. M., *J. Pharm. and Exp. Therap.*, 1911, **2**, 507.

[3] Simonds, J. P., and Brandes, W. W., *J. Immunol.*, 1927, **13**, 1.

[4] Manwaring, W. H., Hosepian, V. M., O'Neill, F. I., and Moy, H. B., *J. Immunol.*, 1925, **10**, 575.

[5] Petersen, W. F., and Levinson, S. A., *J. Immunol.*, 1923, **8**, 349.

[6] Simonds, J. P., and Brandes, W. W., *J. Immunol.*, 1927, **13**, 11.

(1) simplicity, (2) opportunity to repeat and check results, (3) the possibility of assaying any physiologically active substance by comparison with known active agents, (4) opportunity to determine the time relations of the appearance of any active substance in relation to the shock, and (5) opportunity to obtain certain data relative to the nature of the active substance.

Dogs were sensitized by the intravenous injection of horse serum, hog serum, or sheep serum. After an interval of 14 to 28 days, they were anesthetized with ether and sodium barbital, the carotid artery cannulated for recording blood pressure, and the thoracic duct cannulated for collecting lymph. The appropriate shocking dose of serum was given intravenously and the lymph collected for varying periods of time thereafter. This was then tested for physiological activity on a strip of guinea pig's intestine suspended in a bath of Tyrode's solution in the usual manner. In approximately 40% of the experiments run to date (22), the lymph collected after shock had a marked ability to stimulate contractions of the guinea pig intestine, both in the case of normal pigs and in pigs immunized by repeated injection of the antigen used. This activity varied in degree so that in some experiments ¼ cc. of lymph when added to the 200 cc. bath used was effective while in other experiments as much as 5 cc. were required. In no instance did normal lymph, even in doses up to 20 cc. have any such effect. It is interesting to note, however, that the intensity of the shock is not necessarily correlated with the presence of the active substance, because it could be demonstrated in the lymph in a few instances of comparatively weak shocks, while it was occasionally absent in fatal shock.

In a number of experiments, after shock was well developed, and in other cases of fatal shock just prior to death, the chest was rapidly opened and blood aspirated from the inferior vena cava just above the diaphragm. In all instances of fatal shock, and occasionally in non-fatal shock, such blood was shown to be active in stimulating the guinea pig intestine, both of normal pigs and of pigs immunized to the antigen used. In no case did normal pre-shock blood have any such effect, and in only one instance did the femoral vein blood obtained after shock have any activity.

The nature of the active substance appearing in the lymph and in the inferior vena cava blood during anaphylactic shock is at present under investigation.

6132

Effect of Excessive Insulin on the Pancreatic Islets of Young Rats.*

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From the Department of Pathology, Loyola University School of Medicine.

Hypertrophy of the pancreatic islets in diabetes mellitus is usually explained as a compensation resulting from hypoinsulinism. In the thyroid gland experimental evidence has been obtained by Loeb[1] and his associates that excess hormone is inhibitory. Such observations lend strength to the idea that cell regeneration is a response to functional need.

By the method previously used by one of us[2] the mitotic activity of the islet cells was determined in serial sections of the chrome-formol fixed tissue. The results in detail are shown in the table.

TABLE I.

Age at Injection Days	Age when Killed Days	Injections in Units	Mitoses per 100 Islets	Mitoses per 100 Islets in Control
4	7	1(3x)	4.8	32.0
4	7	1(2x), 2(1x)	10.0	
5	7	1(1x), 2(1x)	2.5	28.9
4	9	1(3x), 2(2x)	3.6	20.8
3	9	1(3x), 2(2x)	10.3	
4	9	1(4x), 2(1x)	9.7	
2	9	1(2x), 2(4x)	6.9	
4	10	1(2x), 2(3x)	7.6	
5	10	1(1x), 2(3x)	3.5	8.2
3	11	1(2x), 2(2x)		
		3(1x), 5(1x)	1.8	5.7
4	11	1(4x), 2(1x)		
		3(1x)	3.6	13.8
4	20	1(4x), 2(2x)		
		3(7x), 7(2x)	0.7	3.7

The injections were made at 1 or 2 day intervals with the last and largest dose about 18 hours before autopsy. Usually the pancreases from the 2 or 3 animals were mounted together and from 100 to 800 islets were enumerated. The controls were rats of the same age and they often were litter mates. The insulin treatment inhibited the proliferative activity of the islet cells although the growth of the animals continued normal and their weights were sometimes greater than those of the controls.

* The authors gratefully acknowledge the gift of 1000 units of Iletin (Lilly) from Eli Lilly & Co.

[1] Loeb, L., *J. Med. Res.*, 1920, **41**, 481.

[2] McJunkin, F. A., and Breuhaus, H. C., *Arch. Path.*, 1931, **12**, 900.

6133

Effect of Macerated Kidney on the Mitotic Rate of Kidney Epithelium.

H. C. BREUHAUS AND F. A. MCJUNKIN.

From the Department of Pathology, Loyola University School of Medicine.

Previously we have shown[1] that fresh macerated liver injected in a prescribed way stimulated hepatic regeneration. We have now obtained results which show that by the injection of macerated kidney the mitotic rate of the tubular epithelium may be increased. The stimulant effect appears in the averages. The causes of the individual variations were usually undetermined. The compilation (Fig. 1) includes all treated animals that lived 14 days. Many

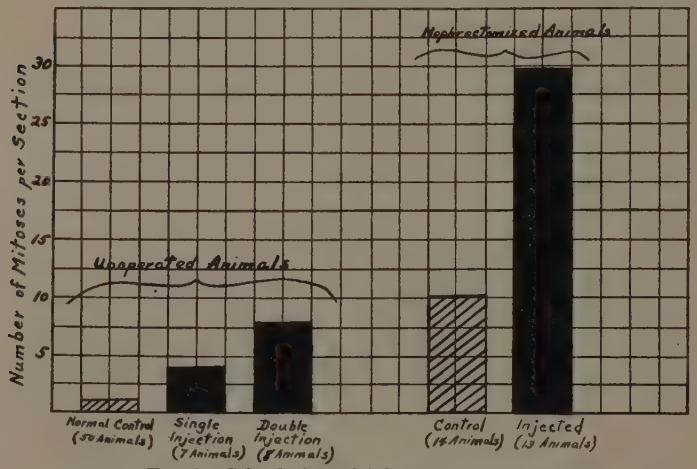

FIG. 1. Stimulation of Kidney Regeneration.

negative results obtained by methods other than those described and not included in this report, are as yet unexplained.

With a few exceptions the experimental rats weighed from 100 to 150 gm. Kidneys from somewhat larger rats were crushed in a mortar for intraperitoneal injection. Seven normal rats were injected with 2.5 gm. crushed kidney and killed on the fourteenth day. Eight other normal animals were injected on the first and fifth day with a total of 4.5 gm. and killed on the fourteenth day. In a second series a unilateral nephrectomy was performed; 14 of these were

[1] McJunkin, F. A., and Breuhaus, H. C., *Arch. Path.*, 1931, **12**, 900.

killed after 2 weeks. Thirteen nephrectomized animals were injected either once on the day after the operation or on the second and fifth day with autopsy on the fourteenth day.

Transverse sections were taken through the middle of the kidney and the number of the mitoses per section determined. The influence of the intraperitoneal injection is shown in the figure. Seven normal rats with one injection had an average of 4 mitoses per section and 8 with 2 injections had an average of 7.6 as compared with 0.75 in 50 normal untreated animals. In a second series the mitotic rate was tripled by the injections with an average of 10 in the nephrectomized and 30 in the injected nephrectomized. At this time we have determined only that the parenteral introduction of the whole cell under the conditions of the experiments increases the mitotic rate of the tubular epithelium.

6134

Absorption of Drugs from the Esophagus.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School.

While studying the effect of acute esophageal obstruction in the dog,[1] it was noted that the production of a closed loop of the esophagus was invariably fatal. Although post mortem evidence indicated that death was due to an aspiration pneumonia or to rupture of the loop with subsequent pleuritis and mediastinitis, the possibility of toxin absorption from the esophageal loop was considered. The following experiments were performed to ascertain the absorptive ability of the esophagus under various conditions.

Dogs were used. Under sodium pentobarbital anesthesia, a closed loop of the esophagus was prepared by ligating the esophagus in the neck and in the thorax just above the diaphragm. The substances to be tested were introduced into the lumen of the esophageal loop and the absorption estimated either by chemical tests of the urine and saliva, or by observation of the characteristic physiological effects.

Five experiments were performed in each group. Potassium iodide (in 10% solution), sodium ferrocyanide (in 10% solution), and sodium nitrite (in 5% solution) were all slowly but definitely

[1] Dragstedt, C. A., and Mullenix, R. B., *Arch. Surg.*, 1932, **24**, 152.

absorbed under the conditions of these experiments. Histamine hydrochloride (in doses up to 3 mg. per kilo), strychnine sulphate (in doses up to 5 mg. per kilo), cocaine hydrochloride (in doses up to 15 mg. per kilo), ephedrine hydrochloride (in doses up to 10 mg. per kilo), nicotine (in doses up to 2 mg. per kilo), and epinephrine hydrochloride (in doses up to 10 mg. per kilo) were not sufficiently absorbed to produce characteristic effects. Phenolsulphonphthalein, methylene blue, and antipyrine were not sufficiently absorbed during the course of 2 hours to be detected in the urine. Distention of the esophageal loop with air pressure up to 60 mm. of mercury after introduction of the above drugs which were not normally absorbed, caused various reflex disturbances of respiration and blood pressure, but did not apparently facilitate absorption. After irrigating the esophageal loop with 50% alcohol, followed by tap water to wash out the alcohol as completely as possible, definite absorption of histamine, strychnine, cocaine, ephedrine, nicotine, and epinephrine was shown. Histamine hydrochloride in doses of 2 mg. per kilo produced an average fall of blood pressure of 30 mm. of mercury in an average time of 15 minutes. Strychnine sulphate in amounts of 1 mg. per kilo produced marked excitability in 3 cases, and convulsions with fatal ending in 2. Ephedrine hydrochloride in amounts of 5 mg. per kilo produced an average rise of blood pressure of 50 mm. of mercury in an average time of 20 minutes. Nicotine in amounts of 2 mg. per kilo produced death in all cases. Epinephrine hydrochloride in amounts of ½ mg. per kilo produced an average rise of blood pressure of 30 mm. of mercury in an average time of 20 minutes.

Distention of the esophageal loop previously irrigated with alcohol with air pressure after introduction of the last group of drugs in general decreased the time necessary to elicit the characteristic effects. For example, the pressor responses after epinephrine developed within one minute when air pressure was used. The increased absorptive ability of the esophagus after irrigation with alcohol gradually decreased with time, to be gone in the majority of cases in 3 hours.

Mammaser and Boyd[2] have noted increased absorption of histamine from the intestine after irrigation with alcohol. Inactivation of histaminase may be considered a factor here. This does not seem a probable factor in the case of the esophagus as, so far as we know, histaminase has not been shown to be present in the esopha-

[2] Mammaser, L. F., and Boyd, T. E., PROC. SOC. EXP. BIOL. AND MED., 1929 **26**, 765.

gus and its absence in the stomach[3] might be considered as indicating that it is not present in the esophagus. The increased absorption of strychnine, epinephrine, nicotine and ephedrine could not be explained on this basis. The increased absorption after alcohol irrigation may be due to removal of mucin. This does not appear to be the case as reintroduction of considerable mucin either with the drugs used or for some interval before introduction of these drugs did not appreciably diminish their absorption. There was no apparent adsorption of the drugs by the mucin. The mucin normally present prior to alcohol irrigation may, however, be distributed so as to act as a mechanical barrier which is not adequately reconstructed by introducing mucin.

Macht[4] has related the low absorbability of the esophagus as compared with the stomach to the histological types of their mucosae.

From our results and those of Macht, it is apparent that the absorptive ability of the esophagus is normally poor, that it is not increased by distention, but that it may be considerably increased by such procedures as lavage with alcohol.

6135

Leucocyte Counts and Bacteremia Studies in Rats Orally Immunized Against Pneumococci.

HERBERT E. MC DANIELS. (Introduced by Lloyd Arnold.)

From the Research Laboratories of the State Department of Public Health and Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine.

Various experimenters[1] have shown that young rats can be vaccinated *per os* against multiple lethal doses of virulent pneumococci. Ross has shown that the blood serum of such orally immunized animals contains antibodies which may be demonstrated by means of the passive immunity conferred upon other animals to which it is transferred. Besides this indication of a general humoral resistance to infection, we have seen no attempts at explanation of the mech-

[3] Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 23.

[4] Macht, D. I., *J. Pharm. and Exp. Therap.*, 1923, **22**, 123.

[1] Killian, H., *Z. f. Hyg. u. Inf.*, 1924, **102**, 279. Eguchi, C., *Ibid.*, 1925, **105**, 74. Kimura, R., *Ibid.*, 1927, **107**, 390. Ross, V., *J. Immunol.*, 1926, **12**, 219. Maëji, Y., *Acta Scholae med. univ. imp.*, Kioto, 1929, **12**, 295.

anism of the immunity of such animals, and it was thought that studies of the leucocyte response, together with blood culture examinations, might throw some light upon the subject.

The method consisted of making periodic tests of the peripheral blood of a series of normal rats and comparing the results with those of a series of rats previously immunized with egg-white and Type I pneumococcus autolysate, as already described.[2] Both sets of rats were inoculated intraperitoneally with 100 fatal doses of the homologous strain of pneumococci. Blood was obtained under sterile precautions from the tail of the animal; examinations were continued as long as the unvaccinated rats survived (2 to 4 days).

From work done on normal persons, it was expected that the leucocyte counts would be subject to wide fluctuations. This proved to be the case. One feature of the leucocyte counts, however, was constant; the immunized animals, after the first 6 hours following injection, showed an appreciably higher white cell count than the unvaccinated controls. This may be attributed to increased mobilization of leucocytes without any considerable diapedesis into the peritoneum. The lower counts in the unvaccinated animals probably reflect the loss of leucocytes which go to make up the exudate in the peritoneal cavity. This loss may go on until a leucopenia occurs, especially if the rats do not die until the third or fourth day.

The growth of pneumococci from 0.1 cc. of blood seeded on blood agar plates was recorded only as negative or positive, with an indication of the amount of growth in the positive cases. All of the unvaccinated controls showed organisms in the cultures within 7 to 12 hours following the inoculation. The amount of growth rapidly rose to 4 plus, the maximum to be obtained. Among the vaccinated animals which survived (96%), none showed a positive blood culture at any time. The remaining 4% of vaccinated animals, which did not survive 100 fatal doses, exhibited an intermediate condition as regards the leucocyte count and the number of pneumococci cultured from their blood; they also lived longer than the controls. While, under the conditions of this experiment, no pneumococci were found at any time in the blood cultures of the animals which survived, it is possible that invasion of the blood stream may have taken place. This may be demonstrable with larger samples of blood.

Whenever there were sufficient pneumococci to be detected in the blood, the outcome was invariably death of the animal.

[2] McDaniels, H. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 587.

6136

Dissociation of Yeast and Bacteria Within the Stomach and Duodenum.

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From the Research Laboratories of the Illinois State Department of Public Health and Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine.

During experiments upon the intestinal absorption of *B. prodigiosus* and various types of yeasts in rats, it was found that many of the plate cultures of the contents of different levels of the gastro-intestinal tract were sterile. To determine whether these plates were actually sterile or whether forms of microbes were present in a phase which did not grow appreciably, we subjected such apparently sterile cultures to the serial plating technic described by Hauduroy.[1] About 0.5 cc. of sterile nutrient broth is taken up in a capillary pipette, the slender part of which is about 3 inches long. After discharging all the broth onto the surface of the plate from which transfer is to be made, the pipette is held so that the side of the Bunsen flame just touches it about 2 inches from the tip. Softening of the glass and the weight of the tip operate to bend the pipette into a spreader which is used to scrape the discharged broth back and forth across the surface of the plate, thereby washing off whatever microbic elements may be present. The same pipette is used to draw up the washings, to discharge them upon the fresh plate, and to spread them over its surface. We have found this technic superior to that of using 2 pipettes and 2 spreaders for this simple operation. Practically all of the apparently sterile plates selected for this study were derived from the stomach or duodenum of the animal; an occasional plate made from the first part of the jejunum was also sterile. These plates were all observed for at least 48 hours (many of them for 60-72 hours) and did not show detectable growth under a hand lens or low power of the microscope.

Table I shows the level from which the material was taken, the strain of yeast used, and the number of the serial transfer in which the first evidence of growth was obtained. The first sign of growth was a dull area over the inoculated part of the plate; smears taken from this area showed large fusiform rods, granular debris, and long square-ended filaments. All of these forms retained the Gram stain either uniformly or in irregular areas. Certain of the growths

[1] Hauduroy, Paul, *C. R. de la Soc. de Biol.*, 1927, **97**, 1392.

TABLE I.

Sample	Level	Yeast	First Sign of Growth
			Transfer
1	Stomach	No. 16	3
2	"	18	2
3	"	17	2
4	"	17	2
5	"	18	4
6	Duodenum	16	2
7	"	17	2
8	"	16	2
9	"	18	5
10	Jejunum	M18	2
11	"	16	3

consisting entirely of the filamentous forms gave rise to typical yeast forms on transfer to acid broth (pH 5.0).

In other experiments, in which *B. prodigiosus* was the test organism, the first appearance of growth was on the fifth serial transfer. This appeared first as a dull area; the next transfer gave more definite evidence of growth in the form of a thicker grayish film. Smears of this material showed spindle-shaped rods with very slender tapering ends; the whole organism being about twice as long as the original "normal" *B. prodigiosus*. These spindle-shaped organisms were amphophilic to the Gram stain. On further serial transfers the appearance of minute colonies was observed under the hand lens; these were flat, round in outline, with smooth margin, and an internal arrangement suggesting a tangled mass of threads. After 10-12 serial transfers the colony size approached more nearly that of the original culture, but the colonies were more raised and glistening. Feeble production of red pigment was the next change to be observed in the subsequent transfers.

In both the yeast and *B. prodigiosus* experiments, litmus lactose agar plates were found to be superior to other plating media in bringing about the transformation to the approximate form and growth characteristics of the original organisms.

6137

Simultaneous Electrograms and Myograms from Isolated Intestinal Segment in Unanaesthetized Dog.

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From the Departments of Surgery and Physiology, University of Illinois School of Medicine.

In a series of dogs, segments of the small intestine 5 to 10 cm. in length were isolated from the remainder of the intestinal tract and transplanted onto the surface of the abdomen. These segments were taken from various portions of the bowel between the upper duodenum and terminal ileum. Continuity of the remainder of the intestinal tract was re-established. When the animals had completely recovered from the operative trauma electrograms were made of the isolated segments. Two electrodes were placed at varying distances apart on the longitudinal axis of the bowel segment and were connected with a string galvanometer. The electrograms obtained were constant in their rate for given segments and reveal some rather characteristic phases. The electrograms persisted after the segment had been completely separated from the central nervous system. They also persisted, although with slight changes in certain phases, whether the segment was visibly active or quiet. This has been discussed more fully elsewhere.[1]

Four dogs have been studied to determine the relationship of respiration and of mechanical activity of the bowel segment to the electrogram. Platinum wire electrodes covered with cotton and saturated with normal saline solution were used. The string of the galvanometer was relatively loose, deflecting 2 cm. per millivolt. A pneumograph was so arranged as to record respiratory movements simultaneously with electrograms on the same film. These showed that there was no relationship between respiration and the electrical complexes obtained from the isolated bowel. (Fig. 1.) Balloons were then inserted into the isolated bowel segments and connected to record contractions of the bowel on the same film with the electrogram. A very small balloon was used and the electrodes were placed on the surface of the bowel directly over it. There was a constant and definite relationship between the mechanical and electrical records. (Fig. 2.) The myogram showed the contraction to begin a brief time after the sharp negative and positive deflection phases of the complex and to correspond to the prolonged

[1] Puestow, C. B., *Arch. Surg.*, 1932, **24**, 565.

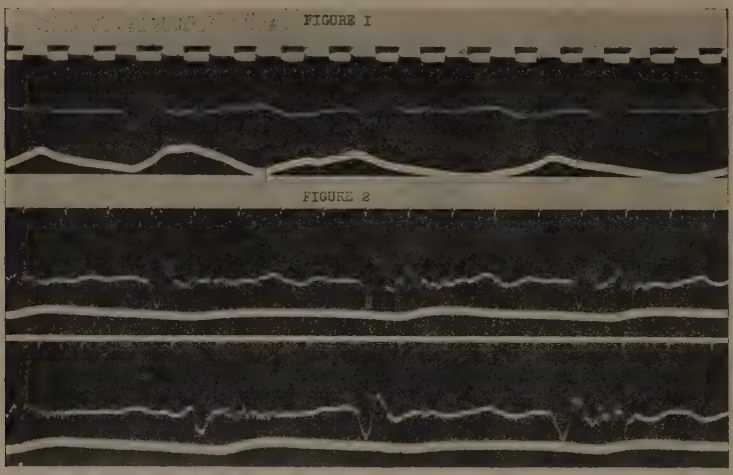

FIG. 1. Simultaneous electrogram of isolated intestinal segment and pneumogram of respiration.
FIG. 2. Simultaneous electrogram and myogram of isolated intestinal segment. Time interval—one second.

phase which shows considerable fluctuation when there is visible activity of the segment. The myograms always were simultaneous with the same phases of the electrogram. Richter[2] obtained electrogastrograms which consisted of 3 to 4 definite phases. He likewise obtained a negative and positive quick phase which precedes the contraction wave and a slow phase which coincides fairly closely with the contraction wave.

6138

Gastrectomy in the Rat.

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Removal of the stomach necessitates anastomosis of esophagus to duodenum; this is made difficult in the dog by the shortness of the abdominal part of the esophagus. In the rodent this part is much longer. It therefore occurred to one of us that such an operation

[2] Richter, Curt P., *Am. J. Physiol.*, 1924, **67**, 612.

* Josiah Macy, Jr., Foundation Fellow.

might be feasible in the rat, provided that some other technique than sewing might be used in making the anastomosis. We had on hand a number of small cannulae made of magnesium and intended for blood-vessel surgery. According to Lexer[1] these were introduced into surgery by Payr. The technique we have developed consists essentially of tying the cut end of the esophagus over one end of the cannula and the end of the duodenum over the other. This unsurgical method could not be expected to work in larger animals, but in the rat it gives very promising results.

We have thus been able to perform gastrectomies on 31 rats, and at present have 8 living. These have survived their operations by 8, 8, 8, 8, 21, 21, 25, 43, and 51 days respectively. Of the 23 that have died, the survival times were 1 (9), 2 (3), 3 (2), 4 (1), 5 (1), 6 (3), 7, 8, 9, 13, and 20 days respectively. In all rats now living the filling of the intestine by way of the anastomosis during the ingestion of an opaque meal has been observed by fluoroscopy. In the accompanying roentgenogram is shown the anastomosis in the rat which is now in its 43rd day; this rat has had acute esophageal obstruction 4 times, but is now able to eat anything it cares for. Four of these rats have regained their pre-operative weights. They begin promptly to eat anything offered them, but lose interest very soon. We have tried feeding by dropper, administration of cod liver oil, and frequent small feedings of varied foods. The blood has not yet been studied, pending the solution of the dietary problem; such studies should be significant in view of the anemias observed by Ivy, Morgan, and Farrell[2] in gastrectomized dogs.

6139

Natural Variability Among White Rats in Degree of Susceptibility to Infection with Eimeria miyairii.*

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From Iowa State College, Ames.

Animals differ in respect to the limits to which infectious organisms can multiply in their bodies and the morbidity of the symptoms.

[1] Lexer-Bevan "General Surgery", 1908, 557.

[2] Ivy, A. C., Morgan, J. E., and Farrell, J. I., *Surg., Gyn., and Obstet.*, 1931, **53**, 1.

* This work was supported by a grant from the Rockefeller Fluid Research Fund at Iowa State College.

The present report is concerned principally with the first generalization in the case of *Eimeria miyairii* in white rats. The individual rats employed were of extremely mixed heredity, involving about 4 strains.

All but 10 (obtained when young from another department) of the 48 rats used in the experiment were raised in our laboratory under conditions which did not permit them to become naturally infected, a fact substantiated by fecal examinations at least every fourth day during the growing period. The growing rats were fed a modification of Steenbock's growth ration. When they had attained a weight of from 65 to 149 gm. (mean wt., 106 gr.; std. dev., 24.06 gr.) they were put individually into specially constructed cages of hardware cloth, which permitted the collection of all the fecal pellets in a pan of water below, and fed, either in a few drops of milk or on bread, 1,500 oocysts daily for 5 days. Seven or 8 days after the first infective feeding the oocysts first appeared in the feces, and continued to appear for from 6 to 8 days thereafter. At the end of this period the discharge of oocysts ceased entirely and the animals could not be reinfected immediately, although we found that a slight degree of reinfection was possible after a lapse of 2 or 3 months.

Our data are based upon daily counts of the total number of oocysts discharged by individual rats during each 24-hour period. The technique is as follows: First, the daily collection of feces from each rat is thoroughly homogenized by an electric mixer. The material is poured into a volumetric flask, and diluted to 200 cc. The content of the flask is poured into a beaker and again thoroughly mixed. A direct count is then made of the number of oocysts in 1.8 cmm. of this suspension, and the total yield for the day is easily calculated. In most cases the yields for the second and third days exceed those for any of the other days. The total number of oocysts discharged by a rat during the period required for the development of total immunity is obtained by adding the daily counts.

The microorganism was originally obtained from the cecum of a wild brown rat. It was passed through 3 white rats, and some of the collection of the last of the 3, in the sporulated condition, was used as the infective material. The number of oocysts constituting the daily dose was arrived at by a process of counting and diluting.

The total number of oocysts passed by the individual rats, with no regard to sex, ranged from 14,100,000 to 169,220,000, with a mean of 54,184,200 $\pm$ 3,213,000, a standard deviation of 33,002,400, and a coefficient of variation of 60.09%. Our frequency dis-

tribution table shows the following classes (yields of oocysts) expressed in terms of $X10^4$ and frequencies (rats) respectively: 2,000, 12; 4,000, 16; 6,000, 9; 8,000, 4; 10,000, 4; 12,000, 2; 14,000, none; 16,000, 1. A separate analysis of the yields for the males gives the following statistical constants: Number, 30; mean, 64,297,675 ± 4,403,100; standard deviation, 35,757,500; coefficient of variation, 55.61%. The same for the females: Number, 18; mean, 37,328,300 ± 2,840,000; standard deviation, 17,864,000; coefficient of variation, 47.86%. The difference of the means for the 2 sexes is 26,969,400; the probable error of the difference, 5,239,000. The ratio of the difference of the means to the probable error of the difference is 5.143, a value which indicates an extremely high degree of significance. It is necessary to be extremely reserved in accepting this sex difference, however, for in most cases the males and females were from different litters and, hence, probably of widely differing heredity. We are now continuing the experiment without splitting up the litters of rats.

Conclusion. White rats show exceedingly great variability in potentiality for the production of oocysts during an infection with *Eimeria miyairii*. If a similar situation exists among other species of either mammals or birds, the implications are far-reaching and important, for they may serve to explain many of the cases where one worker claims to have controlled or cured coccidiosis with a particular food or remedy, while another secures contrary results with the same methods.

6140

Action of Bufagins Isolated from Different Species of Toads.

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In the investigation of *Ch'an Su*, a commercial preparation of the venom of a Chinese toad, and the poisonous secretions of 10 additional species of toads collected from different parts of the world, the authors found variations in the potency, physical constants, and chemical composition of some of the principles that were isolated from them. The secretion of each species of the toads contains at least from 3 to 5 distinct principles that belong to 5 classes of com-

pounds: *cholesterol, epinephrine, bufagin, bufotoxin,* and ***bufotenine.***

Cholesterol occurs in all of the secretions. Spectrographic examinations of several different samples showed the presence of ergosterol in various amounts. It has already been proved that after irradiation the sterol from *Ch'an Su* has an antirachitic action in rats.[1]

Epinephrine occurs in *B. marinus*,[2] *Ch'an Su*,[3] and appears to be present in *B. arenarum, B. regularis, B. formosus,* and *B. bufo gargarizans.* The amount varies from 2 to 5% of the dried secretion.

Bufagin is a word used generically, since it was observed that most of the bufagins, although similar in their action and elementary composition, are quantitatively different from each other as shown by their minimal emetic dose in cats and pigeons, respectively, and also by the average fatal dose in cats (Table I). Besides, their

TABLE I.

Bufagin from	m. p.	Elementary Analysis		Minimal Emetic Dose in Pigeons	Minimal Emetic Dose in Cats	Average Fatal Dose in Cats
		C	H			
	°C.	%	%	mg. per kg.	mg. per kg.	mg. per kg.
Ch'an Su	222-223	70.02	7.67	0.300	0.125	0.23 (27)*
Bufo marinus	212-213	71.87	8.07	0.375	0.300	0.58 (20)*
B. arenarum	220	69.68	7.96	0.150	0.060	0.09 (11)*
B. viridis viridis	255-255.5	70.65	8.33	0.150	0.060	0.11 (10)*
B. valliceps	205-206	70.41	8.46	0.200	0.080	0.21 (10)*
B. formosus	253-254	70.87	8.37	0.150	0.070	0.10 (10)*

* Figures in parentheses indicate the number of cats used for the determination of the dose.

melting points are not the same throughout. The bufagins possess the essential features of the members of the digitalis group. The action of the bufagins is not persistent, for the cat can eliminate completely a minimal emetic dose within one to few hours. The cat unit of each principle is less than 1 mg. All of the bufagins raise blood pressure and stimulate the isolated intestines and uterus. Locally, they produce numbness of the tongue. The bufagin from *B. marinus* was first isolated by Abel,[2] and that from *B. formosus* by Kotake,[4] and Wieland and Vocke,[5] respectively.

[1] Chen, K. K., Jensen, H., and Chen, A. L., *J. Pharm. Exp. Ther.*, 1931, **43**, 13.

[2] Abel, J. J., and Macht, D. I., *J. Pharm. Exp. Ther.*, 1911-12, **3**, 319.

[3] Jensen, H., and Chen, K. K., *J. Biol. Chem.*, 1929, **82**, 397.

[4] Kotake, M., *Liebig's Ann.*, 1928, **465**, 11; *Sc. Pap. Inst. Phys. Chem. Res.*, 1928, **9**, 233.

[5] Wieland, H., and Vocke, F., *Liebig's Ann.*, 1930, **481**, 215.

6141

Action of Bufotoxins.

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From the Lilly Research Laboratories, Indianapolis, and Johns Hopkins Medical School, Baltimore.

Wieland and Alles[1] isolated bufotoxin from the skin of *B. vulgaris* or *B. bufo bufo*. In our study of *Ch'an Su* and the secretions of 10 additional species of toads, we obtained a bufotoxin from the dried venom of each species. The word bufotoxin is used generically for the same reason as given for bufagins. Chemically, the bufotoxins are formed by the conjugation of one molecule of suberyl-arginine with one molecule of a specific bufagin found in the respective secretions. Their pharmacological action is similar to that of bufagins, being different only in degree (Table I), as shown by the

TABLE I.

Bufotoxin from	m. p.	Elementary Analysis C	H	N	Minimal Emetic Dose in Pigeons	Minimal Emetic Dose in Cats	Average Fatal Dose in Cats
	°C.	%	%	%	mg. per kg.	mg. per kg.	mg. per kg.
Ch'an Su	200	64.05	8.31	7.60	0.200	0.125	0.36 (9)*
Bufo bufo bufo	202	60.91	8.21	7.39	0.200	0.125	0.30 (10)*
B. marinus	200	63.09	7.98	7.55	0.200	0.125	0.38 (10)*
B. arenarum	194-195	61.80	7.95	7.61	0.200	0.150	0.41 (13)*
B. bufo gargarizans	195-197	63.54	8.08	7.78	0.250	0.125	0.49 (10)*

* Figures in parentheses indicate the number of cats used for the determination of the dose.

systolic standstill in the myocardiogram of frogs, typical changes in the electrocardiograms of cats, and emesis in cats and pigeons. Locally, they are bitter to the taste.

[1] Wieland, H., and Alles, R., *Ber. deut. chem. Gessel.*, 1922, **55**, 1789.

6142

Action of Bufotenines.

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From the Lilly Research Laboratories, Indianapolis, and Johns Hopkins Medical School, Baltimore.

Handovsky[1] isolated an "alkaloid" from *B. vulgaris*, and named it bufotenine. In our investigation of *Ch'an Su*, we[2] obtained cinobufotenine, and found it to have a marked pressor action. It appears now that the secretions of most toads contain bufotenines—a name also used generically. The bufotenines are organic bases having an indole ring, and form organic salts such as the flavianates, which have been used in this investigation. Three bufotenine flavianates, prepared from *Ch'an Su* and the secretions of *B. fowleri* and *B. bufo gargarizans*, respectively, have a marked pressor action in pithed cats (Table I); those separated from the secretions of *B. formosus*, *B. bufo bufo*, and *B. viridis viridis* are slightly less active; and the remaining bufotenines have little blood pressure raising property. All of them have an oxytocic action, and with one exception, a stimulating action on isolated intestines.

TABLE I.

Bufotenine flavianate from	m.p.	Elementary Analysis C	H	N	S	Pressor Activity
	°C.	%	%	%	%	
Bufo viridis viridis	170	49.03	4.82	9.70	—*	65
B. formosus	186-187	49.65	4.77	9.54	5.94	89
B. valliceps	261-262	49.03	3.85	11.14	6.57	5
B. arenarum	130-131	47.70	4.71	10.36	5.74	5
B. fowleri	188-189	50.24	4.85	9.91	6.00	108
B. alvarius	224-225	49.30	4.94	9.62	5.81	3

* Not determined.

[1] Handovsky, H., *Arch. Exp. Path. Pharm.*, 1920, **86**, 138.

[2] Chen, K. K., Jensen, H., and Chen, A. L., *J. Pharm. Exp. Ther.*, 1931, **43**, 13.

6143

Quantitative Method for Determination of Precipitin in Small Volumes of Rabbit Anti-Crystalline-Egg-Albumin-Serum.

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The usual dilution methods for determining the precipitin titer of antiserum only roughly approximate the relative antibody strength of a given serum and give no information with regard to the absolute quantity of antibody. Analysis of precipitates from the precipitinogen-precipitin reaction for their nitrogen content by the micro-Kjeldahl method has been used by Wu and his collaborators[1] and Heidelberger and Kendall.[2] Precise measurements of the antibody per unit volume of antiserum, and exact studies on the ratio of antigen to antibody in the precipitate have thus been made possible. This method requires at least 0.5 cc. of serum for each test and cannot be used when only small volumes of antiserum are available.

We have found that the following procedure gives satisfactory results for determining the quantity of precipitin in a small volume of rabbit anti-crystalline-egg-albumin serum. The method was suggested by figures presented by Heidelberger and Kendall. It depends on the determination of the amount of antigen necessary to precipitate the precipitins completely from a given volume of an antiserum. This determination of the mg. of antigen necessary completely to precipitate the antibodies from a sample of such serum is easily accomplished without analytical methods because the point of maximum precipitation proves to be that point where antigen as well as antibody is completely precipitated and neither antigen nor antibody remains in the supernatant fluid. We have called this optimum ratio of antigen to antibody the neutralization point. Furthermore, at this optimum point antigen and antibody combine in a ratio of about 1:13. The description of the method will clarify these points.

Method. The stock solution of crystalline egg albumin was diluted with saline to provide known concentrations of the antigen expressed in mg. of nitrogen per cc. One-tenth cc. of each antigen dilution was mixed with an equal volume of the antiserum tested,

[1] Wu, H., Chang, L. H., and Li, C. P., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 853. Wu, H., Sah, P. P. T., and Li, C. P., *Ibid.*, 1929, **26**, 737.

[2] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1929, **50**, 809, and *Science*, 1930, **72**, 252.

and incubated 2 hours at 37°, then put in the icebox overnight. The next day, after centrifugation, the supernatant fluids over the precipitates were tested for antigen and for antibody. When antigen in excess had been added to the anti-egg-albumin-serum, antigen only was found in the supernatant fluid, while antibody only could be demonstrated in the supernatant fluids of the tubes in which the quantity of antigen added was insufficient to precipitate all the antibody. If the dilutions of antigen were properly spaced, the supernatant fluid of one tube would be found to contain neither antigen nor antibody within the limits of delicacy of the test. This represented our neutralization point.

The importance of the neutralization point depended on the facts that: (1) the amount of precipitate obtained at the neutralization point proved to be the maximum amount of precipitate obtainable from the antiserum and (2) the ratio of antigen to antibody in this precipitate was a constant ratio of about 1:13. That the quantity of antigen necessary to establish the neutralization point represented the amount necessary to precipitate the maximum amount of antibody was proved by carrying out micro-Kjeldahl determinations for the nitrogen content of the precipitates obtained by incubating 1 cc. portions of antiserum with equal quantities of the same antigen dilutions used in the small test tube experiments described above. That the ratio of antigen to antibody at the neutralization point is relatively constant was proved by determining the mg. of antibody precipitated by antigen at the neutralization point in a large number of sera from different animals. The results indicated: (1) the identity of the point of neutralization and the point of maximum precipitation, and (2) a constant ratio of about 1:13 between antigen and antibody in the precipitate obtained at the neutralization point. Mg. of antigen at neutralization point X 13 = Mg. antibody in serum.

TABLE I.
Identity of points of neutralization and maximum precipitation.

Serum cc.	Antigen Mg. N	Precipitate Mg. N	Substance in excess
1	.031	.358	Antibody
1	.037	.456	Antibody
1	.043	.556	Neither
1	.050	.491	Antigen
1	.056	.319	Antigen

TABLE II.
Ratio of antigen to antibody at neutralization point.

Serum No.	Antigen Mg. N	Antibody Mg. N	Ratio: Aby/Agn.
1	.008	.104	13.0
2	.008	.121	15.1
8	.025	.329	13.1
19	.040	.441	11.0
20	.040	.534	13.3
25	.048	.577	12.0
31	.060	.770	12.8
32	.060	.819	13.6